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- IMPROVING FOOD

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Preface

The present PhD thesis is a result of a project entitled: 'Low-fat dairy products - microstructure, sensory properties and consumer perception', carried out at The Royal Veterinary and Agricultural University, Department of Food Science, from April 2003 through April 2006, under the supervision of associate professor Richard Ipsen (principal supervisor) and assistant professor Michael Bom Frøst (co-supervisor). The project has been interdisciplinary in nature, integrating dairy technology and sensory science, and was funded by The Danish Research Council for Technology and Production Sciences and the Danish Dairy Research Foundation - Danish Dairy Board.

I wish to extend my sincere gratitude to everyone who has contributed to this endeavour, including all co-authors as well as the remaining staff at Dairy Technology and Sensory Science, Department of Food Science, The Royal veterinary and Agricultural University (KVL). Our industrial partners Arla Foods Ingredients, Arla Foods Innovation and CP Kelco contributed not only excellent products, but also invaluable advice for the present project; their participation is gratefully acknowledged.

Abstract

The dairy industry is in need of rationally mastering the development of low-calorie products in order to meet present and future demand from the market. So far the industry has employed two approaches to achieve the desired reduction of fat and carbohydrate: 1) using fat replacers, *i.e.* ingredients with a lower caloric density, but mimicking the sensory and functional properties of those they substitute, and 2) technological modifications, *i.e.* adapting manufacturing processes such that the products acquire the desired properties, but without adding special ingredients. The objective of the present project has been to elucidate relationships between the microstructure of a set of low-fat, semi-solid dairy products and their sensory properties. The product range comprised stirred yoghurt, cream cheese and acidified milk drinks, covering a continuum from highly structured materials without defined fracture properties to almost Newtonian fluids. The products were submitted to descriptive sensory analysis, generating sensory maps suitable for multivariate data analysis, as well as to several instrumental analyses, *e.g.* confocal laser scanning microscopy (with appropriate extraction of image features), rheological (conventional as well as novel techniques) and sprectroscopic methods (fluorescence, NIR and NMR). Using descriptive analysis we have generated sensory vocabularies pertaining to each product group studied, and comprising both descriptors related to flavour and aroma as well as texture-related descriptor (oral, tactile and visual). In particular we have emphasized the compound descriptor creaminess, due to its special relevance to low-fat products, which are expected by consumers to match corresponding full-fat products in terms of creaminess.

Dansk sammendrag (abstract in Danish)

Mejeriindustrien har et behov for på rationel vis at beherske udviklingen af produkter med at lavt energiindhold for at imødegå både nutidige og fremtidige markedskrav. Industrien har hidtil benyttet sig af to forskellige måder til at opnå den ønskede reduktion af fedt: 1) brug af fedterstattere, dvs. ingredienser med lavere energitæthed, men med sensoriske og funktionelle egenskaber lig dem de erstatter, og 2) modifikation af fremstillingsteknologien, dvs. en ændring af forarbejdningen således at produkterne opnår de ønskede egenskaber, men uden at tilsætte specielle ingredienser. Målet med nærværende projekt har været at undersøge sammenhænge mellem mikrostrukturen i en række halvfaste lavfedt-mejeriprodukter og disses sensoriske egenskaber. De undersøgte produkter var røreyoghurt, flødeost og syrnede mælkedrikke, som til sammen dækker spændet fra højt strukturerede materialer uden egentlige brudegenskaber til næsten-Newtonske væsker. Produkterne blev undersøgt ved deskriptiv sensorisk analyse, hvilket frembragte sensoriske "kort" egnet til multivariat dataanalyse, såvel som ved en række instrumentelle metoder, bl.a. confokal mikroskopi (med tilhørende *feature extraction*), diverse reologiske (både konventionelle og nyere teknikker) og spektroskopiske metoder (fluorescens, NIR og NMR). Ved hjælp af den deskriptive analyse har vi frembragt sensoriske vokabularer til hver produktgruppe, omfattende både lugt- og smagsrelaterede deskriptorer og deskriptorer relateret til tekstur (oral, taktil og visuel). Vi har lagt særlig vægt på at at afdække betydningen af den sammensatte deskriptor cremethed, da det betragtes som særligt vigtigt for lavfedt-produkter at opnå en cremethed der matcher den som fuldfedtprodukter har.

Resumen en castellano (abstract in Spanish)

La industría lácteo necesita dominar el desarrollo racional de productos con baja densidad calórica para poder responder a los requisitos presentes y futuros del mercado. Hasta ahora, la industría se ha aprovechado de dos maneras distintas de lograr la reducción deseada de grasa: 1) la utilización de sustitutos, o sea ingredientes de menor densidad calórica, pero de una empeno sensorial y funcional a los componentes que sustituye, y 2) la modificación tecnológica, o sea adaptar el procesamiento de manera que los productos obtengan las caracteristicas deseadas, pero sin agregar ingredientes especiales. El objeto del presente proyecto ha sido estudiar las relaciones entre la microestructura de un conjunto de productos lácteos ligeros y semi-sólidos, y sus propiedades sensoriales. Los productos estudiados fueron yogur batido, queso crema y bebidas lácteas acidificadas, formando un continuo desde materiales altamente estructurados, mas sin propiedades de fractura definidas, hasta lo quasi-Newtoniano. Los productos fueron sometidos a análisis descriptivo (sensorial), lo cual generó mapas sensoriales aptos para analisis multivariado, asi como también a una serie de métodos instrumentales, incluyendo la microscopía confocal (CLSM, con la extracción correspondiente de *features*), varios métodos reológicos (tanto los más convencionales como algunos novedosos) y espectroscópicos (flourescencia, NIR y NMR). Mediante el análisis descriptivo se logró generar vocabularios sensoriales pertinenetes a cada categoría de productos, incluyendo tanto términos de sabor y aroma como términos relacionados a la textura (oral, de tacto y visual). Hemos puesto especial énfasis en la semántica del término cremosidad, ya que es considerado esencial para productos ligeros lograr una cremosidad comparable con la de productos convencionales.

Papers

I: Frøst, M.B. and T. Janhøj. Individual differences in creaminess perception in a sensory panel studied by multiway methods. Submitted to *Food Quality and Preference*.

II: Janhøj, T., C.B. Petersen, R. Ipsen and M.B. Frøst (2006) Sensory and rheological characterization of low-fat stirred yogurt. *Journal of Texture Studies* **37** (3) 276-299.

III: Janhøj, T. and R. Ipsen (2006) Effect of pre-heat treatment on the functionality of microparticulated whey protein in acid milk gels. *Milchwissenschaft* **61** (2) 131-134.

IV: Janhøj, T., C.M. Andersen, N. Viereck, M.B. Frøst, R. Ipsen, and S. Edrud (2006) Sensory, rheological and spectroscopic characterization of low-fat cream cheese. *Proceedings of the 4th International Symposium on Food Rheology and Structure, February 19-20, 2006, Zürich*, p. 383-387.

V: Rasmussen, M.A., T. Janhøj and R. Ipsen. Effect of fat, protein and shear on graininess, viscosity and syneresis in low-fat stirred yoghurt. Accepted for publication in *Milchwissenschaft*.

VI: Johansen, S.M.B., J. Laugesen, T. Janhøj, R. Ipsen and M. B. Frøst. Relationship between visual sensory properties and surface images in plain yoghurt and cream cheese. To be submitted to *Food Quality and Preference.*

VII: Johansen, S.M.B., T. Janhøj, J. Laugesen, R. Ipsen, M.B. Frøst. Prediction of sensory properties of semi-solid dairy products from confocal laser scanning micrographs using global feature extraction and multivariate regression techniques. Manuscript.

VIII: Janhøj, T., Frøst, M.B. and R. Ipsen. Sensory and rheological characterization of acidified milk drinks. Manuscript.

Nomenclature, abbreviations

AMT	Angle Measure Technique
ANOVA	Analysis of Variance
APLSR	ANOVA Partial Least Squares Regression
CLSM	Confocal Laser Scanning Microscopy
FCP	Free Choice Profiling
FFT	Fast Fourier Transform
φ	Volume fraction
G'	Elastic modulus
GDL	Glucono-δ-lactone
GLCM	Grey Level Co-occurence Matrix
HB	Herschel-Bulkley
IDF	International Dairy Federation
LV	Latent Variable
MA	Mean Angle
MDY	Mean Difference in Y
MLR	Multiple Linear Regression
MPP	Microparticulated Protein
MSNF	Milk Solids Non Fat
MWP	Microparticulated Whey Protein
NIR	Near Infrared
NMR	Nuclear Magnetic Resonance
NPLS	N-Way Partial Least Squares
PARAFAC	Parallel Factor Analysis
PC	Principal Component
PCA	Principal Component Analysis
PL	Power Law
PLSR	Partial Least Squares Regression
QDA	Quantitative Descriptive Analysis
R^2	Coefficient of Determination, Explained Variance
RMSECV	Root Mean Square Error of Cross-Validation
RSM	Response Surface Methodology
SMP	Skimmed Milk Powder
SSSA	Spectral Stress Strain Analysis
TPA	Texture Profile Analysis
TPM	Texture Profile Method
UTM	Universal Testing Machine
WHC	Water Holding Capacity
WPC	Whey Protein Concentrate

1 Introduction

While low-fat dairy products may appear to be a relatively contemporary phenomenon, the dairy industry (especially in Denmark) actually has a long history of manufacturing these; for example, all cheese produced in Denmark pre-1920 was low-fat as milk fat was reserved for butter production. But low-fat products have only taken off commercially in the past two decades, in response to an increasingly health-conscious public. Innovation in this field has been on the rise for the same period of time. A reflection of this is the number of hits for the term "low-fat" in the bibliometric database Web of Science, as well as the patent database Derwent:

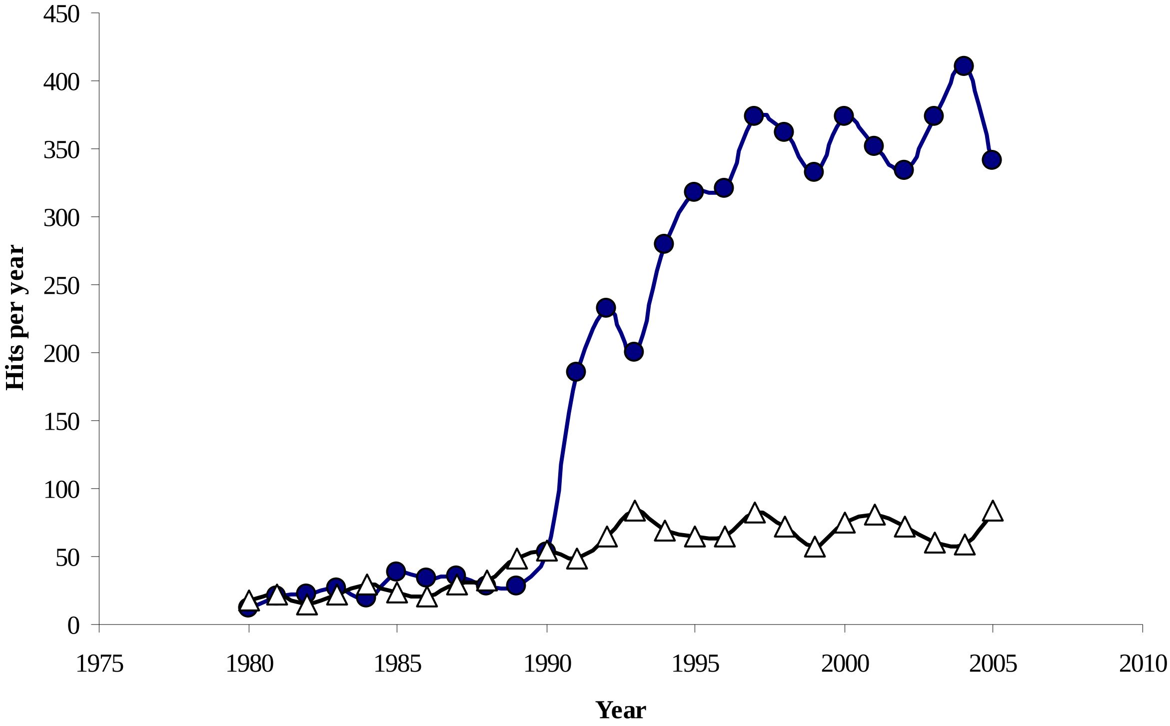

Figure 1 Hits per year on the search term *low-fat* in the bibliometric database Web of Science (●), and the patent database Derwent Innovations Index (Δ).

Even if we take into account the general increase in publication activity in food science in the period considered, it is evident that something very significant happened around 1990; it is also clear that there is no sign of abatement. For patents the development has been more steady.

As many as 75 to 90% of all new food products launched fail in the market (Buisson, 1995). Sensorially, many of the first reduced-fat products to enter the market have left much to be desired, and low-fat products in general have suffered from a bad image among consumers. They are commonly perceived as bland, and to have 'something missing'. In one study, low-fat yoghurt and fluid milk were evaluated more favourably than low-fat cheese and low-fat spreads (Knox *et al*., 2001). Thus, technological challenges abound for the dairy industry, especially in mimicking the flavour and texture profiles of full-fat products. The problems encountered by product development staff have been studied using qualitative methods (Parr *et al*., 2001). Problems with mouthfeel/texture, flavour, changes to production process, shelf life as well as confusion with regards to which ingredients to use were mentioned as barriers to the development of low-fat spreads and yoghurts. For cheese, the flavour and the change of production process was perceived as less of a problem; low sales volume, and consequently inefficient production, in addition to moutfeel/texture as well as fat functionality were deemed more problematic. Attitudes and technological competence of product development staff towards low-fat products was also mentioned as a barrier.

The present project has been exploratory in nature, which is why the present dissertation appears to have a more meandering course than most; as such, it has had a less clear-cut objective than the majority of PhD projects. For the sake of convenience, we have often resorted to marketing our efforts as 'The Creaminess Project', but our purpose have in fact been somewhat wider, namely to gather knowledge of low-fat dairy products, from the microscopic level through human perception to consumer attitutes to product quality. Still, perceived creaminess has been found to be positively correlated to consumer liking for a wide range of dairy products, *e.g.* in fresh and reconstituted milks and creams (Richardson-Harman *et al*., 2000), in vanilla puddings (Elmore *et al*., 1999), in yoghurts (Folkenberg and Martens, 2003; Ward *et al*., 1999) and ice cream (Lähteenmäki and Tuorila, 1994); this warrants closer scrutiny of the concept. Largely following the same approach, the project can be seen as a successor to the work of (2001), which dealt with fattiness perception in fluid milk. Both projects have been centered around sensory descriptive analyses of dairy products, complemented with an assortment of instrumental methods: rheological, spectroscopic, imaging, and others. Not accounted for here is a series of consumer perception studies on the same products[1].

Texture researchers seem to be largely divided in two groups. One group studies texture from a general perspective, while the other group tends to focus on the sensory perception of one particular food category. The line of thought of the first group is clearly deductive, whereas the other is more abductive.

[1] The results of these studies will appear in a series of papers by M.B. Frøst.

To paraphrase Alina Szczesniak[2], the present work is best described as 'commodity work', *i.e.* it clearly belongs to the latter group. We do not take that as a slight but rather tend take the view that serious texture research can be performed on actual foods, and studying just one food category at a time.

The present work has been centered around a series of trials on low-fat dairy products: yoghurt, cream cheese and acidified milk drinks. For the sake of brevity, these trials will henceforward be referred to as **Trial Y**, **Trial CC** and **Trial AMD**. The relationship between these trials is illustrated below:

Table 1 Relationships between sensory trials and papers.

Topic	Trial Y	Trial CC	Trial AMD
Sensory-instrumental relationships	**Paper II**	**Paper IV**	**Paper VIII**
Spectroscopy		**Paper IV**	**Paper VIII**
Confocal microscopy	**Paper VII**	**Paper VII**	**Not covered**
Surface imaging	**Paper VI**	**Paper VI**	**Not covered**
Individual differences	**Paper I**	**Not covered**	**Not covered**

Paper III and **Paper V** do not relate directly to the sensory trials. These papers address techological, non-sensory issues: the effect of microparticulated whey protein particles on the properties of acid milk gels and the factors influencing the formation of graininess in stirred yoghurt.

[2] »Following the breakthrough in the 1960s and 1970s in surfacing the multi-parameter nature of texture and in defining the general principles of texture acceptability, the field has essentially reverted to commodity work«
(Szczesniak, 2002).

2 Range of products studied

Textural properties of low-fat cheese have been reviewed in the literature (Banks, 2004; Drake and Swanson, 1995). In the present study we have been concerned with an intermediate range of dairy textures upwards limited by, and not including, natural cheese (a solid with defined fracture properties), and downwards by, and not including, liquid milk (a Newtonian fluid), the latter being the subject of the of Frøst (2002). Basically, dairy products (or at least a majority of them) can loosely be characterized structurally as follows:

Table 2 Some properties of main categories of dairy products

Fluid milks	**Semi-solid products**	**Cheese**
O/W emulsions	Acid milk gels (mostly)	Aged (rennet) gels
Newtonian	Non-Newtonian fluids	Solids which may or may not fracture

2.1 Stirred yoghurt (Trial Y)

Stirred yoghurt is commonly made from a heat-treated and homogenized milk base, inoculated with a starter culture, and, after the fermentation, submitted to mechanical treatment. The sensory properties of the stirred yoghurt depends on a variety of techonological factors: fat and protein level (the latter can be increased by concentration of addition of milk protein, either bulk ingredients such as skimmed milk powder, whey protein concentrate, or more specialized ingredients such as microparticulated whey protein), type of starter culture, heat-treatment temperature and time, homogenization pressure, fermentation temperature, among others (Sodini *et al*., 2002). Structurally, stirred yoghurt is a concentrated suspension of acid milk gel particles in serum, forming a weak gel with or without a defined yield stress. Compared to other dairy products, the scientific literature on yoghurt and other fermented milk products is quite extensive. The reference product, to which all low-fat products are compared, is the full-fat variety (with 3.5% fat in Denmark). Fat replacement is often achieved by increasing the protein level. Several milk protein ingredients have been employed in this study.

2.2 Cream cheese (Trial CC)

The starting material for cream cheese manufacture is cream or a mixture of milk and cream. This mix is pasteurized and homogenized, and inoculated with a starter culture (Kosikowski and Mistry, 1997; Phadungath, 2005). Cream cheese is made by concentration of an acid cheese curd, traditionally by straining in nylon bags, or more recently by centrifugal separation at an elevated temperature. It is thus a spreadable (*i.e.* with a considerable yield stress), concentrated suspension of acid milk gel particles, stabilized by hydrocolloids. It is mostly used as a sandwich spread, in salads/dips and in cheesecake. There is little in the way of scientific publications for the technology this category of dairy products, with most techological know-how being kept in-house by manufacturers. In the United States, cream cheese must contain at least 33% fat; a low-fat variety, Neufchatel, contains 20-33% fat. In this study (where the fat levels have been much lower: 0-9%) we have varied the chemical composition (fat, salt, pH) rather than adding fat replacers.

2.3 Acidified milk drinks (Trial AMD)

Acidified milk drinks is a diverse group of fluid drinks comprising drinking yoghurts (made by dilution of a fermented yoghurt base) and milk-juice drinks (fruit juices with added milk powder). A *sine qua non* for these products is stabilization, mostly with pectins. Structurally they are dilute, *quasi*-Newtonian, suspensions of milk solids in an acidified (either by fermentation or chemically) milk medium, stabilized and thickened by hydrocolloids, the type and addition levels of which are varied in this study. These products are inherently low-fat. Being a recent addition to the palette of dairy products, there is little published so far. Because of the low viscosity, turbulence is likely predominant during oral processing of these products.

Table 3. Colloidal properties of dairy product categories included in this work.

Stirred yoghurt	Cream cheese	Acidified milk drinks
Meta-stable suspension of acid milk gel particles pH 4.3	Concentrated, stabilized suspension of acid milk gel particles pH 4.4 to 5.0	Dilute, stabilized suspension of acid milk gel particles pH 4.0

3 Milk gel systems: microstructure and sensory properties

3.1 Microstructure and rheological properties of milk gels

Over the past 15 years, milk gels (acid milk gels as well as thermally set whey protein gels) have been studied extensively as models of solid and semi-solid dairy products. Almost all milk gel studies make use of rheological methods, a few involve microscopy (successively migrating from electron microscopy to confocal laser scanning microscopy); recently a handful of studies involving sensory properties of milk gels have appeared (Gwartney *et al*., 2004; Pereira *et al*., 2003). For the sake of reproducibility, most of the acid milk gel studies are on reconstituted low-heat skimmed milk powder, with glucono-δ-lactone (GDL) as an acidulant, even though the kinetics of acidification is different from that of starter cultures (Lucey *et al*., 1998). Some studies have dealt with the effect of fat on the microstructure of acid milk gels.

3.1.1 Filled milk gels

A subset of milk gels studies have been concerned with filled gels (van Vliet, 1988), and have introduced the concept of active vs. inactive fillers, a subject of particular pertinence to low-fat dairy technology. Active fillers, *e.g.* recombined milk fat globules (*i.e.*, containing casein at the o/w interface) interact with the gel matrix; increasing the volume fraction φ of the filler phase will generally strenghten the gel, *i.e.* increase the elastic modulus *G'*. Inactive fillers, by contrast, *e.g.* washed natural milk fat globules, do not interact with the gel matrix, and consequently weaken the resultant gel: the elastic modulus decrease with increasing volume of filler phase. These concepts originate in the polymer field; filled milk gels have been studied since the late 1980s, again mostly by rheological methods, and predominantly on thermally set whey protein gels, which are more well-defined than casein gels, and furthermore can be altered structurally in several ways. The elastic modulus *G′* has furthermore been found to depend on the mean particle size d_{32} of the dispersed phase, with ln *G′* being a linear function of d_{32} (Dickinson and

Chen, 1999). The higher the elastic modulus of the gel matrix (*i.e.*, the continous phase), the less the effect of the active filler and *vice versa*. Computer simulations, which fortunately corroborate experimental findings, have shown that small active filler particles have a bigger effect on gel strength than larger ones (Wijmans and Dickinson, 1998). By contrast, among inactive filler particles, only very large ones influence the gelation process. The mechanical properties of the filler particles have also been found to be of importance. For rigid glass particles suspended in aqueous gellan solutions G' exhibited a minimum at $\varphi = 0.20$, hinting at interaction between the particles, whereas for suspensions of deformable gellan bead G' was found to decrease linearly with φ, which might be due to compliance of the deformable particles under stress (Jampen *et al*., 2001). Employing fractionated milk fat (from very low-melt to very high-melt) is thus an alternative way of modulating the rheological properties of thermally set filled whey protein gels (Mor *et al*., 1999; Mor-Rosenberg *et al*., 2004; Rosenberg, 2000).

These results are fundamental in *e.g.* yoghurt manufacture, where the milk base is commonly submitted to a pre-heat treatment (in which whey proteins denature and combine with casein micelles) and subsequently homogenized, resulting in an integration of the milk fat and milk protein phases. The end result is a firmer yoghurt, more resistant to syneresis.

3.2 Sensory studies on milk gel model systems

Only a handful of milk gel studies so far have taken to evaluating milk gel models sensorially. One problem with GDL-gels is the long acidification time at a relatively high temperature; gels from non-heat treated, reconstituted low-heat skimmed milk powder are probably not fit for human consumption. Indeed, the reconstituted milk is commonly preserved with potent preservatives such as sodium azide prior to acidification.

3.2.1 Sensory properties of acid milk gels

In one study on GDL-acidified acid milk gels with (10-20 % total solids (TS), heat treated at 90ºC, 30 min./non-heat treated) eleven non-oral descriptors including firmness, smoothness and cohesiveness were evaluated (Pereira *et al.*, 2003). Instrumental measurements comprised a back extrusion test, syneresis evaluation and confocal laser scanning microscopy (CLSM). Heat treatment turned out to the main factor in providing the textural difference between the samples. Smoothness could not be discerned among samples from non-heat treated milk. Confocal micrographs could be related qualitatively to sensory and functional properties; the density of the gel network increased considerably with TS, with a more pronounced interconnectivity between protein clusters in gels made from heat treated milk. In another study, milk gels (10-20% TS, 0-4% fat) were produced by fermentation of aheat treated, reconstituted milk using a yoghurt starter culture (Pereira *et al*., 2006); one could argue that this is not properly a model milk gel, since a starter culture was used. Oral descriptors were included in the descriptive analysis (but no flavour or aroma descriptors), and five parameters were quantified from the confocal micrographs (mean cluster size, mean cluster numbers, mean end point numbers, mean pore size and mean pore numbers). To the rheological tests were added dynamic oscillation (frequency sweep). Increasing the fat content was found to cause a decrease in mean pore size, and an increase in mean cluster size. Sensorially the fat caused the gels to become firmer, creamier and more cohesive and sticky. The 1st principal component of the sensory data (spanning 93.8% of the variation in the sensory data) was regressed on the 1st, 2nd and 3rd principal component of the instrumental data and image parameters (R^2=96.3%; not a very standard data analytical procedure). Using only image parameters gave a considerably poorer model (R^2=47.2%) than a model based on only instrumental data (R^2=86.9%). The lower R^2 for the image parameters was ascribed by the authors to the heterogeneity of the confocal micrographs. (An alternative explanation could be that the rheological methods capture the dynamics of the structure breakdown during oral processing whereas the image parameters only express the static microstructure of the milk gels).

3.2.2 Sensory studies of whey protein gels

Whey protein gels are of particular interest because their microstructure can be manipulated precisely by altering pH and salt addition. The sensory properties of whey protein gels (12% protein) filled with sunflower oil (0-20%), and either with a stranded or particulate microstructure, have been studied (Gwartney *et al.*, 2004). Gelation was induced by heating at 80ºC/30 min. Apart from a descriptive analysis, again limited to texture descriptors, water holding capacity (WHC) and fracture properties (determined by torsion gelometry) were determined. The main sensory effect of increasing lipid level was an increase in number of chews required to (*chew-down properties*) due to increased fracture stress. The remaining descriptors, related to prefracture and first bite and including smoothness, firmness, and crumbliness, were related to gel type (stranded/particulate).

4 Fat replacement in dairy products

Two fundamental strategies exist for fat replacement: 1) ingredient solutions (utilization of fat replacers) and 2) optimization of processing parameters (of particular relevance to cream cheese and other cheeses). A brief rundown of the issue of fat replacement using different ingredients will be given here.

4.1 Fat replacers and mimetics

Fat replacers are required to emulate the sensory qualities of fats: appearance, flavour, aroma and texture (Jones, 1996; Lucca and Tepper, 1994; Sandrou and Arvanitoyannis, 2000). Fat has a considerable impact on flavour release, causing a retardation of the release of flavour compounds from the food matrix; in low-fat products flavour release tends to be faster. Apart from that, fat, and especially milk fat, imparts a flavour of its own. Texturally, fat plays a role depending on whether it acts as an active filler or not. Fat replacing ingredients can be fat-like (*e.g.* Olestra, a sugar polyester), carbohydrate- or protein-based. Carbohydrate- and protein-based fat replacers are more properly termed fat mimetics because of their more limited range of applications (essentially because they can't be used as cooking or frying oils).

4.1.1 Microparticulated proteins as fat mimetics

Simplesse® was the first microparticulated protein to be approved as a fat mimetic. The microparticles are produced by a controlled denaturation of protein at high-shear conditions. The kinetics of microparticle formation from whey proteins has been studied extensively (Spiegel, 1999a; Spiegel, 1999b; Spiegel and Huss, 2002). The current Simplesse® is whey protein based; earlier versions included egg white protein (Singer and Moser, 1993). The functionality of microparticulated proteins such as Simplesse® is to behave like a creamy fluid by virtue of their uniform spherical shape and small particle size (~ 0.1-3.0 μm (Singer, 1990); smaller particles lack 'substantialness' whereas larger particles are perceived as gritty (Singer, 1996)). In addition, in the right concentration it ostensibly plays a role as a structure breaker, in much the same manner as fat globules do in gelled systems. In a different interpretation of the functionality of fat mimetics (principally carbohydrate-based), the fat mimetic gel particles rotate relative to each other under shearing conditions, providing a fluidity of the mass of particles whose lubricating, 'ball-bearing' effect could be thought of mimicking the rheological and surface properties of fat globules (Tolstoguzov, 2003). Among the cited dairy applications of Simplesse® are natural and processed cheeses, cream cheese, ice cream, acidified milk drinks, sour cream, cottage cheese dressing and fluid milk. The patent covering the invention of Simplesse® (Singer et al., 1988) expired in 2005, and much activity in this field is expected in the coming years (Oestergaard, 2005).

A number of studies (referenced in **Paper III**) have dealt with the effect of microparticulated protein on the microstructure and texture of dairy products. In **Paper II** we have documented that microparticulated milk protein is capable of producing a significantly *higher* creaminess in a 0.3% fat stirred yoghurt than a full-fat yoghurt reference sample. In **Paper III** he have investigated the mechanism of functional parameters such as firmness and water holding capacity in an acid milk gel model system. The microparticulated whey protein was added to the milk base before or after heat treatment at 90ºC/min. We found no discernible difference in neither firmness nor water holding capacity, leading to the conclusion that the microparticulated whey protein does not act as an active filler in an acid milk gel. This is in marked contrast to homogenized, heat treated milk fat globules in fermented milk products; it is thus evident that the microparticulated proteins actually work sensorially, but in a different manner that the milk fat globules they replace.

5 Studying food texture

Without getting into a lenghty discussion over the meaning of the term texture, a brief clarification is appropriate here. By texture we mean the sensory manifestation of food structure; it is thus a strictly sensory term (Szczesniak, 2002). This definition lumps oral, tactile, visual and auditory terms together, and is merely what is left over from taste and aroma (the chemical and trigeminal senses). In a stricter sense, visual and auditory properties are excluded, but, in any case, texture perception does not originate from a single tactile sense. Etymologically, texture is derived from he Latin verb *texo*, to weave, from which is also derived the noun *textile*. An alternative meaning of the term is used in the field of image texture, which will be dealt with later.

5.1 Sensory analysis of food texture

5.1.1 Descriptive analysis

Descriptive analysis has been termed 'the most sophisticated tools available in the arsenal of the sensory scientist' (Lawless and Heymann, 1998). It comes in a variety of shapes, including the competing trademarked methods Quantitative Descriptive Analysis™ (QDA) and Spectrum™. A special adaptation for texture characterization is the so-called Texture Profile Method (TPM), dating from the early 1960s. In practise, generic descriptive analysis developed *ad hoc* for a specific task, and incorporating elements of the above methods, are used (Murray *et al*., 2001). Common to these methods is the use of trained panellists, the application of a randomized serving order (to remedy biases), and the evaluation (scaling) of descriptors in the sensorially correct order, starting with visual appearance and ending with after-mouthfeel.

In Quantitative Descriptive Analysis™ the relative differences between products are quantitated. As the attributes are not scored in absolute terms, it is unwise to compare results between different panels, or over time. By contrast, the Spectrum™ method pretends to produce absolute scores, which enables comparisons between panels, or over time. For this reason, Spectrum™ panellist need appreciably more training. To calibrate the intensities, reference products are used. This is problematic because these reference products are not globally available and are reformulated more or less frequently. The Spectrum™ is developed from the Texture Profile Method, which, as the name indicates, only deals with texture characterization. The problem with this restriction is that the results may be biased by the dumping effect (Lawless and Heymann, 1998). Omitting descriptors (in the case of TPM: all non-texture descriptors) from the ballot may bias one or more of the remaining descriptors. For this reason we have taken to characterize our trial products exhaustively, instead of restricting ourselves to characterizing texture properties.

Another salient feature of TPM is the use of fixed vocabulary lists. However, rather than relying on a fixed vocabulary we have developed sensory descriptors for each of the three trials in this project. Still, reference materials were used whenever feasible.

Table 4. Sensory descriptors used. Descriptors marked by an asterisk are dynamic in nature, *i.e.* require movement to be evaluated.

	Trial Y	**Trial CC**	**Trial AMD**
Appearance			
	Whiteness	*Glossy*	*Glass coating*
	Green	*Grain concentration*	*Curtains*
	Grey	*Grain size*	*Transparency*
	Yellowness	*White*	*Visual viscosity*
	Glossy	*Grey*	*Colour*
	Grainy surface	*Blue*	
Aroma (smell)			
	Tomato	*Cream*	*Buttermilk*
	Lamb	*Butter*	*Raspberry*
	Creamy	*Acidic*	*Boiled milk*
	Buttermilk	*Old milk*	
	Flour	*Goat*	
Flavour			
	Lamb	*Cream*	*Sweet*
	Butter	*Butter*	*Buttermilk*
	Cream	*Goat*	*Raspberry*
	Buttermilk	*Salt*	*Cream*
	Floury	*Sour*	*Citrus*
	Sour	*Sweet*	*Boiled milk*
	Sweet		

Texture/mouthfeel			
	*Oral viscosity**	*Oral firmness**	*Straw resistance**
	*Smoothness**	*Meltdown rate**	*Oral viscosity**
	*Meltdown rate**	*Smoothness*	*Smoothness**
	*Astringent**	*Grainy**	*Floury*
	Fatty after-mouthfeel	*Floury*	*Astringent**
	Dry after-mouthfeel	*Chalky*	*Fatty after-mouthfeel*
		*Sticky**	
		After-mouthfeel	
		*Astringent**	
Non-oral manipulation			
	*Non-oral viscosity**	*Tactile resistance*	
	Grainy on lid		
	Viscosity by spoon		
	Flow from spoon		
Meta-descriptor			
	Creaminess	*Creaminess*	*Creaminess*

It should be noticed that the sensory descriptors in Table 4 are actually translated from Danish[3].

It is noticeable that several descriptors such as *Astringent*, *Sweet* are repeated in each trial, forming a base vocabulary. Other descriptors are more product-specific; *Salt* appears only in **Trial CC**, as neither yoghurt nor the acidified milk drinks contain any salt. On the texture side we notice that yoghurts and acified milk drinks can be characterized in terms of *Oral viscosity*, while *Oral firmess* is more appropriate for cream cheese.

The descriptor C*reaminess* was used differently from the others:

1. The very use of the descriptor was imposed by the panel leader.
2. No consensus on the use of the term *Creaminess* was sought between the panellists. Indeed, the panellists were instructed to use their own concept of *Creaminess*.
3. No reference material was provided for *Creaminess*.

All three items violate the principles of descriptive analysis to varying degrees. Moreover, the very concept of asking a panellist to assign a score of a complex descriptor such as *Creaminess* is actually a violation of the simple psychophysical model underlying all sensory science (Lawless and Heymann, 1998).

The rationale behind these choices was that we were interested in identifying the sensory dimensions underpinning the concept of *Creaminess*; it would thus be less useful to impose an *a priori* definition of the term.

One fundamental limitation of descriptive analysis methods in texture research is their lack of ability to account for the dynamics of texture perception, *i.e.* changes over time (Wilkinson *et al*., 2000). A general texture dynamics model with the three dimensions degree of structure, degree of lubrication and time has been developed with the objective of

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[3] Translating sensory descriptors is a perilous undertaking. An amusing example of this is found in a well-known multilingual texture word list (Drake, 1989). The English term *creamy* is translated into Danish *flødeagtig*, which literally means *cream-like*. The Danish informant thus manages to misinterpret the English term completely, even though he must have been conscious of the context in which the word is used in a texture lexicon.

providing a starting point for a general theoretical framework for the psychophysics of mastication (Hutchings and Lillford, 1988). Each food is thus thought of having a unique breakdown path. Time intensity methods, where one single descriptor is recorded as a function of time, or progressive profiling, where a small set descriptors are scored successively over time, are, in theory, more suitable for this purpose. However, several key sensory descriptors in this study (marked by an asterisk in Table 4) are dynamic in nature, i.e. they require movement to be assessed.

5.1.2 Analysis of descriptive analysis data

Descriptive analysis lends itself marvellously to multivariate data analysis (Dijksterhuis, 1995). This can actually be seen as a problem as it is all too easy to gather data and even easier to misinterpret it (Dijksterhuis and Byrne, 2005). These methods are capable on reducing the dimensionalty of data tables, and displaying the relationship between several variables at a time, but at the expense of resorting to latent variables, which may or may not be easy to interpret.

One of the best remedies to ensure model consistency is replication; ideally, sensory trials should be performed on material produced in, say, three replicates. This will give a clear estimate of the consistency of the manufacture of the materials. In **Trial Y** we have produced stirred yoghurt in triplicate - this was necessary because of the inherent instable character of stirred yoghurt (no gelatin or other stabilizer was added), and because of the fact that all testing had to be performed on exactly seven day old samples. In **Trial CC** and **Trial AMD** we have not replicated the samples (or just a few, in the case of **Trial CC**), but we did perform the sensory and instrumental measurements in triplicate. This enabled us to quantitate the measurement uncertainty precisely. Another important means of ensuring the validity of descriptive analysis data is cross-validation, which is particularly useful in guarding against overfitting, which is always a danger in latent variables modelling (Martens and Martens, 2001).

Regardless of which data analytical strategy is followed, elucidation of the causal relationships between sensory descriptors can be difficult; and even more difficult for sensory-instrumental relationships.

5.1.3 Trial Y

The sensory analysis of **Trial Y** on stirred yoghurt is presented in **Paper II.** Three descriptors were found to be ineffective in separating between the samples, namely *Tomato aroma*, *Buttermilk aroma* and *Grey colour*. These were consequently omitted from the subsequent Principal Component Analysis:

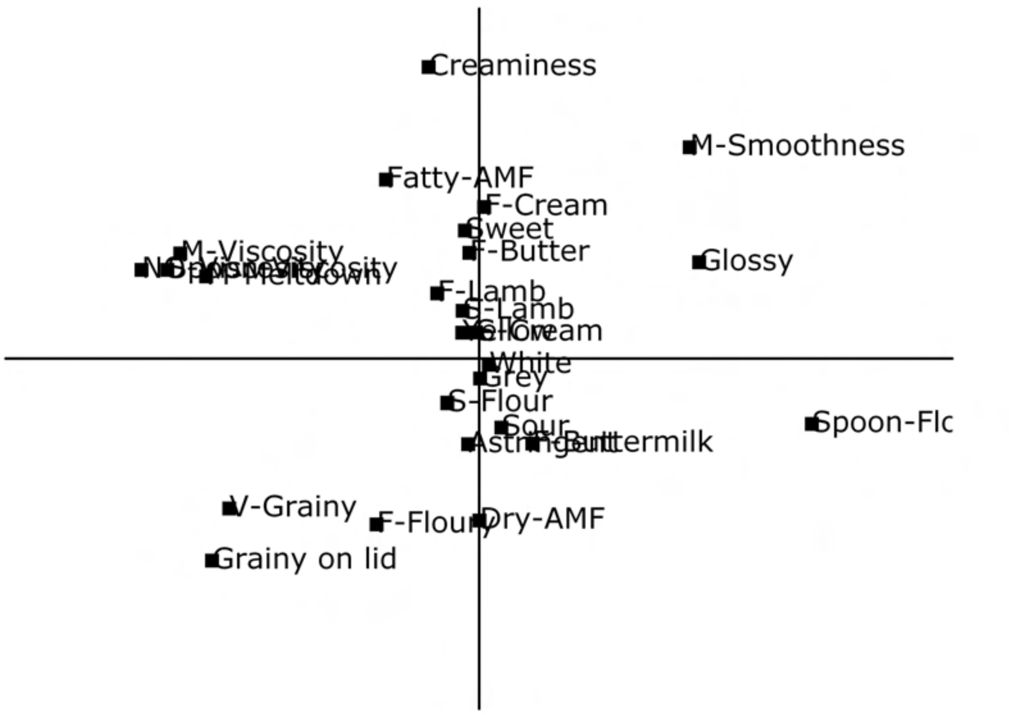

Figure 2 PCA loadings plot of two first PC, Trial Y.

A three-PC model accounted for 63+21+4=88 per cent explained variance. Interestingly, *Creaminess* turned out to be the most important variable. From the scatter plot of *Creaminess* vs. *Oral viscosity* we see that initially the two are linearly related, but eventually a plateau is reached. A the highest levels of Oral viscosity the *Creaminess* even seems to decrease.

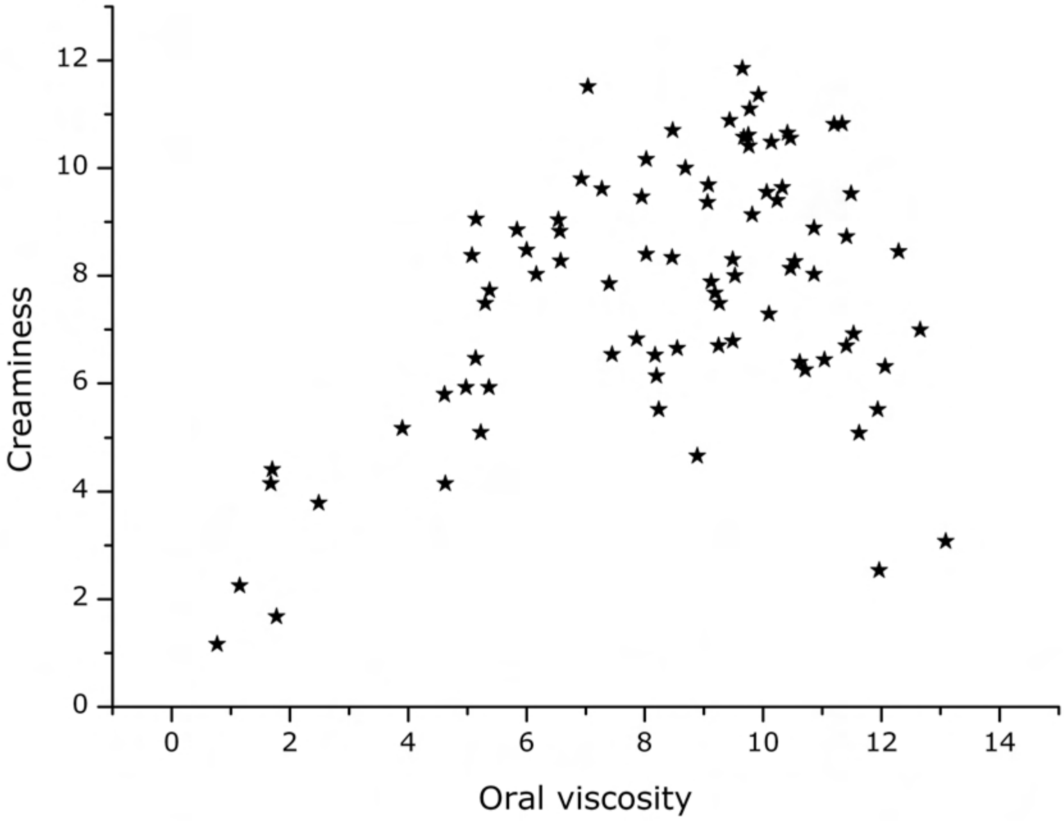

Figure 3. Scatter plot of *Creaminess* vs. *Oral viscosity*, Trial Y.

However, one should be cautious when interpreting this relationship. It is clear that there is a positive correlation between *Oral viscosity* and *Creaminess*, but the subsequent fall could be due to underlying (lurking) variable. For instance, Oral viscosity increases with the design variable Protein level, which also tends to increase graininess. The increased graininess could be the cause of the decreasing *Creaminess* at high *Oral viscosity*.

5.1.4 Trial CC

The sensory analysis of this trial is documented in **Paper IV**. Two descriptors, *Grain concentration* and *After-mouthfeel* could not discriminate the products in an ANOVA and were thus omitted.

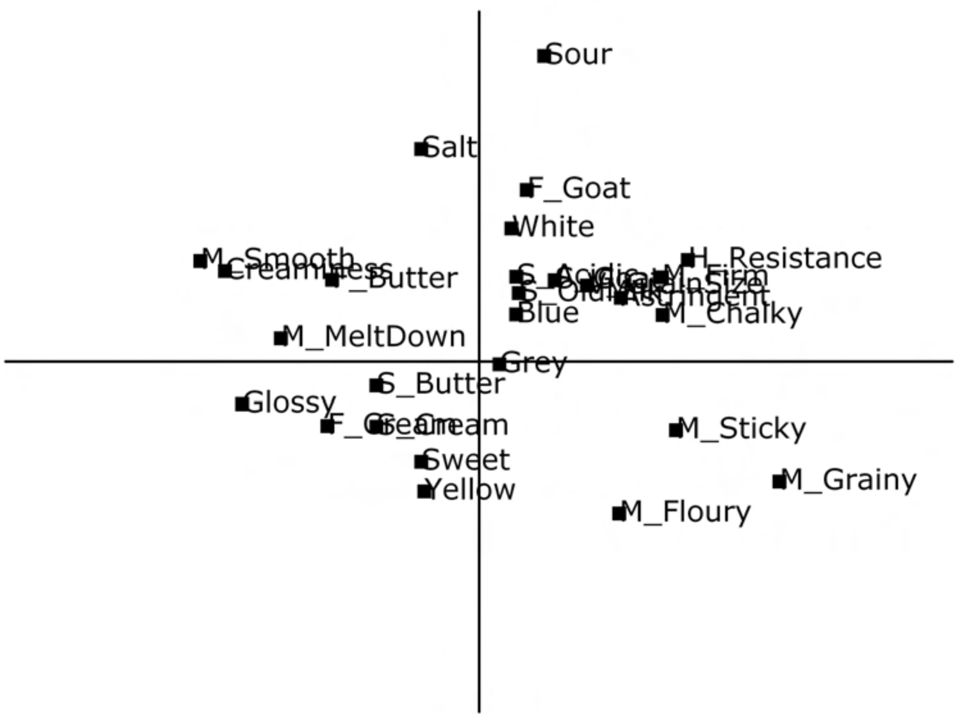

Figure 4. PCA loadings plot of first two PC, Trial CC.

We notice that *Creaminess* appears close to, and is thus postively correlated to, *Oral smoothness*, *Butter flavour*, and opposite to/negatively correlated to *Oral graininess*, *Oral flouriness* and *Oral stickiness*. The actual correlations are not always immediately evident from the loadings plot. For instance, we find the following correlation coefficients between *Creaminess* and *Hand resistance*: -0.94; *Oral firmness*: -0.91; *Oral smoothness*: 0.98; *Oral graininess*: -0.98

The loadings plot present the relationship between the descriptors, but elucidating the actual causal relationship between them is fraught with pitfalls, a good example of which is found in the scatter plot of Creaminess vs. Oral firmness.

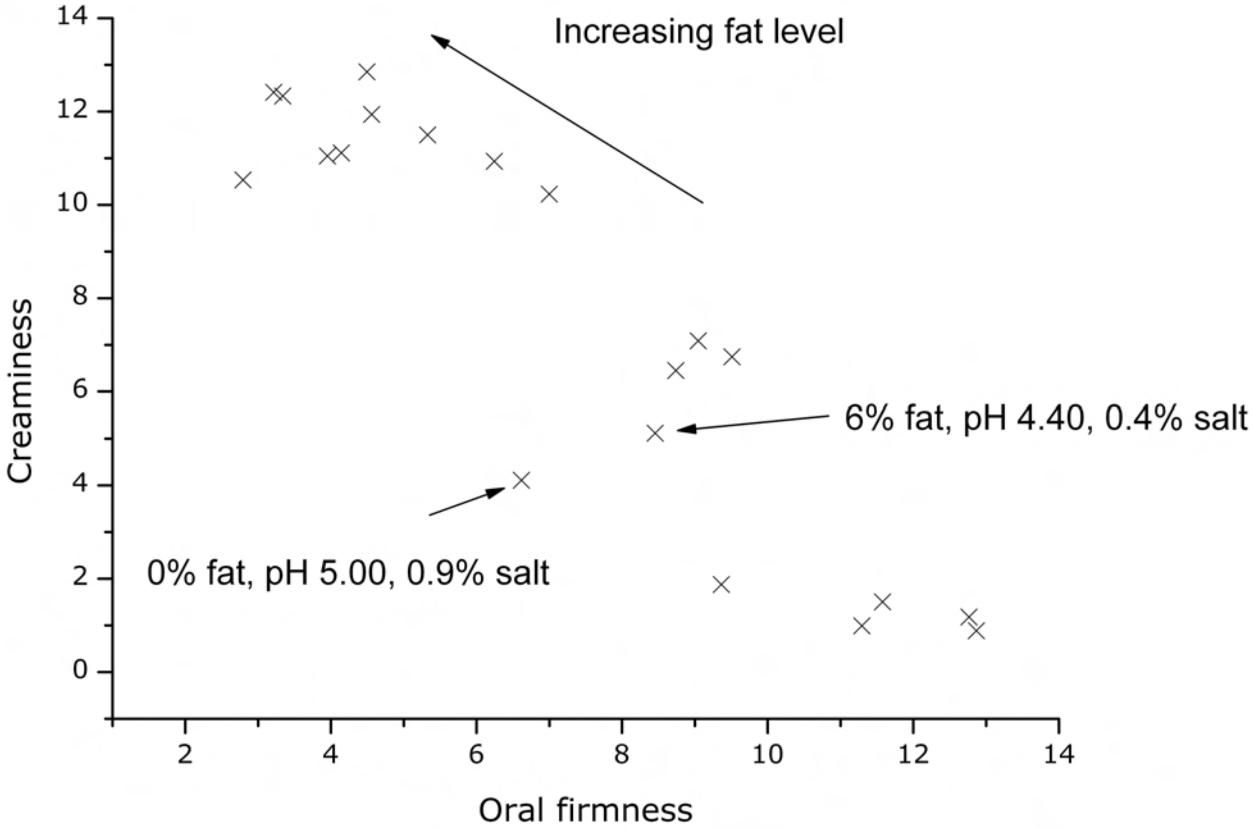

Figure 5. Scatter plot of *Creaminess* vs. *Oral firmness*, Trial CC.

Surprisingly we find *Oral firmness* and *Creaminess* to be negatively correlated, which is the very opposite of the result found in **Trial Y**. This leads us to look for a *lurking variable* (Weisberg, 2005), *i.e.* we surmise that the correlation between *Oral firmness* and *Creaminess* is spurious or non-causal. An important clue is provided in by the location of two samples the plot of *Creaminess* vs. *Oral firmness,* namely 1) the sample with 0% fat, pH 5.00 and 0.9% salt and 2) the sample with 6% fat, pH 4.40 and 0.4% salt. The samples do not follow the general trend of a decrease in *Oral firmness* with fat level. Instrumental particle size data (**Paper VI**) confirm that the former had a considerably smaller mean particle size than the latter. Indeed, if we plot *Creaminess* vs. *Oral graininess* we find:

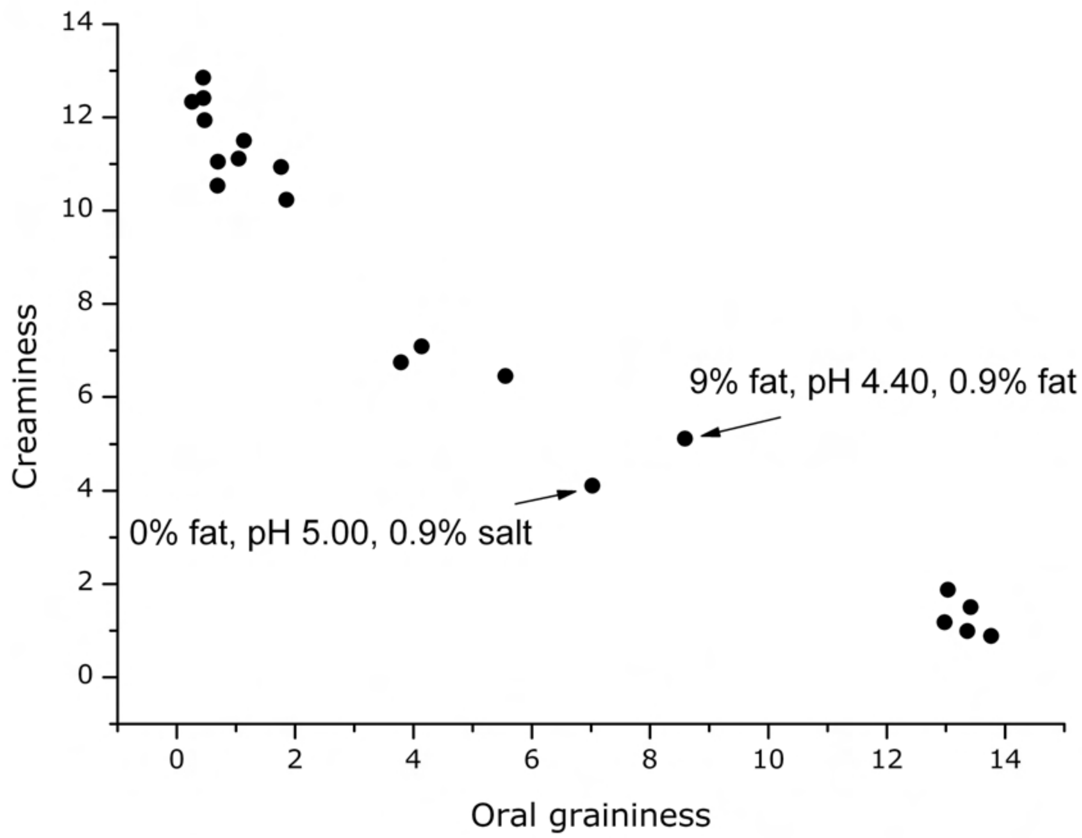

Figure 6. Scatter plot of *Creaminess* vs. *Oral graininess*, Trial CC.

What seems to be at play here is the electrostatic aggregation of acid milk gel particles in the cream cheese. Cheeses with low pH (close to iso-electric pH) and low salt are evidently grainier than cheeses with high pH and high salt, which makes perfect sense; fat inhibits the aggregation of cheese particles, for reasons that are not entirely clear. In **Paper V** the effect of fat protein and mechanical treatment has been studied, using stirred yoghurt as a model. Post-aggregation of acid milk gel particles was found to correlate positively to protein level and negatively fat level to mechanical treatment. The effect of pH on texture of cream cheese and Neufchâtel cheese (retail brands adjusted with NH_3) has been studied (Aliste and Kindstedt, 2005). It was found that firmness decreases with increasing pH. Because of the lurking variable *Oral graininess*, this effect is difficult to discern in our system.

The conclusion of this part is that *Creaminess* is completely governed by the particle size and its sensory manifestation *Oral graininess*. This opens several avenues for product development in the low-fat cream cheese area, further optimizing pH, salt and, of course, fat.

5.1.5 Trial AMD

The sensory analysis of this trial is documented in **Paper VIII**. One descriptor, *Curtains* could not discriminate the products in an ANOVA and was thus omitted.

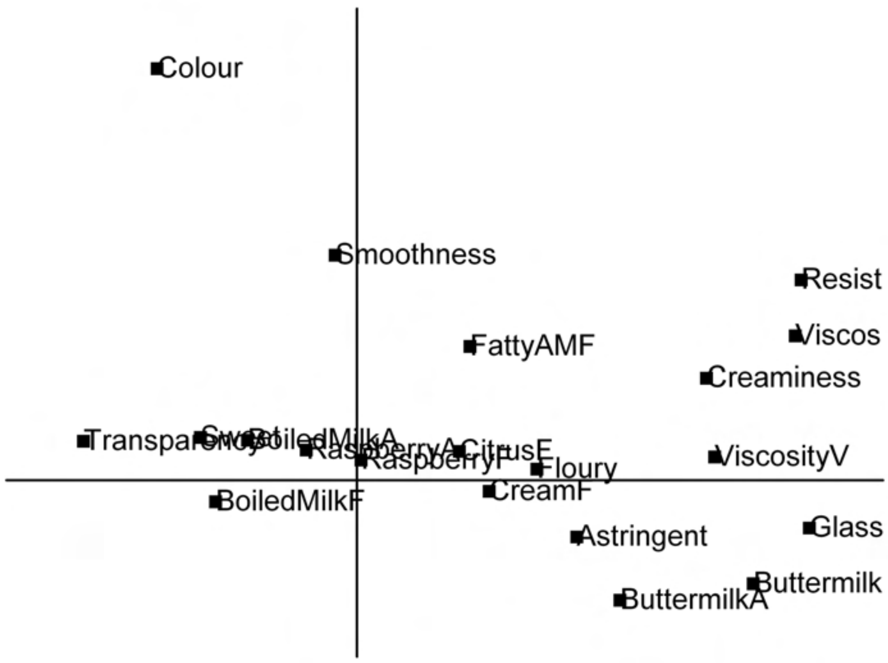

Figure 7. PCA loadings plot of two first PC, Trial AMD.

Closer scrutiny of the data shows that *Creaminess* depends on *Smoothness* in a complex manner, depending on the milk solids level. The correlation is positive in milk drinks with 3% milk solds non fat (MSNF), but negative in drinks with 8% MSNF. The relationship with *Oral viscosity* is more straightforward, with a very high positive correlation (r = 0.97).

5.1.6 Individual differences

Since the location of products (scores) and variables (loadings) are often of more interest in descriptive analysis than the relationships between the way the individual panellists use the scales, the data are commonly averaged over panellists prior to multivariate data analysis, *e.g.* PCA. One way to analyze the differences between panellists is to use three-way decomposition or regression methods, of which PARAFAC and NPLS are examples. In **Paper I** we have used these methods to explore how the panellists differ in perception of the descriptors. Alternatively, each panellist can be modelled individually by PCA and PLS. However, with multiway methods a common underlying model for all panellists is assumed.

5.2 Studying food texture instrumentally

5.2.1 Psychorheology: the sensory relevance of rheology

It took less than a decade from the coining of the term *rheology* until *psychorheology* came about, by the joint forces of an applied rheologist, G.W. Scott Blair, and a psychologist, David Katz (Scott Blair, 1949). Psychorheology was meant to be the link between rheology and sensory perception. Katz had been active in the dough field already in the 1930s, and Scott Blair was working on the sensory perception of curd firmness. In the 1970s there were high hopes that psychorheology eventually would provide a grand unified theory linking rheology and sensory perception (Drake, 1979). It is telling that the *Journal of Texture Studies* carries the subtitle *An International Journal of Rheology, Psychorheology, Physical and Sensory Testing of Foods,* even though the term psychorheology is little used today (five hits on the bibliographical database Web of Science since 1980 hardly makes it a red-hot research area).

There has been much debate about which shear rate is prevalent in the mouth, not least because of the practical relevance (predictive purposes) of the issue. One of the most important results in this area has been the so-called ideal curve (Shama and Sherman, 1973). According to this, the characteristic shear rate of a given food depends on its flow characteristics.

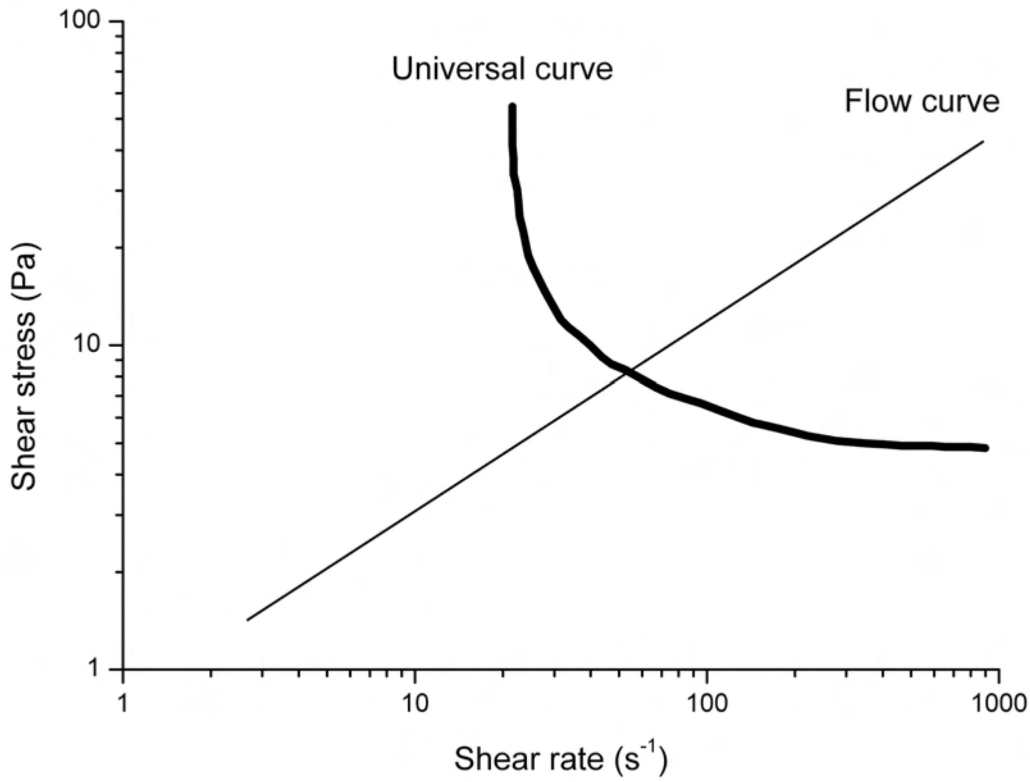

Figure 8. The Ideal curve (schematic). The most relevant shear rate is found at the intersection between the flow curve and the universal curve.

For yoghurt, the relevant shear rate should be around 50 s^{-1}. This is merely an abstraction, as it is inconceivable that one single shear rate should predominate throughout the oral cavity. The flow pattern in the mouth has recently been modelled numerically (Mathmann *et al.*, 2006), but so far only for Newtonian materials.

5.2.2 Imitative methods: TPA

Out of the 1960s came the still widely used instrumental Texture Profile Analysis, in which the sample is submitted to a cyclical movement (Friedman et al., 1963). The TPA is the prototypical imitative texture measurement technique as it attempts to imitate the the mehanical processing in the mouth. The force is recorded continuosly, and several texture parameters are derived from the force curve, including hardness, cohesiveness, elasticity, adhesiveness, brittleness, chewiness, gumminess and viscosity. As such, the method claims to deliver sensorially relevant data on all imaginable food types - a prime example of deductive scientific reasoning. TPA has been severely criticized for being methodologically inconsistent (Peleg, 1983); the results depend greatly on sample size and the testing conditions (*e.g.* per cent deformation), and all the postulated texture parameters are given in force units. For these reasons, the use of TPA in cheese has been discouraged (Walstra and Peleg, 1991), and it has not been used in this work. Given the pervasive technological optimism of the 1960s, the notion of assessing the texture of foods by means of a single measurement on an instrument is perhaps not so far-fetched, but it is telling that Dr. Malcolm Bourne as late as in the mid-1970s felt the need to emphasize the limited scope of rheology in explaining the sensory perception of texture (Bourne, 1977).

5.2.3 Shear viscometry.

Shear viscometry, using a rotational viscometer, is the reference large-strain rheological method for semi-solid foods. Three different measurement geometries have been used to record flow curves in this study: a cup-and-bob system (**Trial Y**), a parallel plate system (**Trial CC**) and a double gap system (**Trial AMD**). For **Trial CC**, shear viscometry proved untenable for non-fat samples. The raw data, shear stress/apparent viscosity, is used directly to model sensory data.

5.2.4 Dynamic oscillation

In dynamic oscillation, the same equipment as in shear viscometry is used to measure elastic and viscous moduli in the linear visco-elastic region, *i.e.* at small deformations. The moduli characterize the gel network; their relation to sensory properties are thus more indirect than viscometry data. There have some claims in the literature that small-deformation data correlate better to sensory viscosity (Skriver *et al*., 1999; Stanley and Taylor, 1993), but as mentioned in **Paper II**, moduli and shear viscosities are often correlated, and difficult to separate causally. Dynamic oscillation proved untenable for the very weakly structured acidified milk drinks in **Trial AMD**[4].

5.2.5 Elongational viscometry

A couple of relatively novel rheological techniques have been used in the present study, namely squeezing flow viscometry (**Trial Y**; **Paper II** and **IV**) and contraction flow viscometry (**Trial CC**; **Paper IV**). Ideally, both methods measure elongational properties (and are, as such, fundamental rheological methods), which might be more relevant sensorially than shear (van Vliet, 2002), since mastication resembles a squeezing flow between parallel plates; however, this has yet to be validated by sensory data. The fundamental problem with elongational viscometry is the difficulty of producing a purely elongational flow field, without shear flow. Other sensory situations where elongational flow might be more relevant are sucking a fluid through a straw and speading a semi-solid material with a knife.

Squeezing flow viscometry originates in the polymer field (Chatraei *et al*., 1981), and has more recently been introduced to food rheology by Peleg and co-workers (Campanella and Peleg, 2002). Normally an Instron Universal Testing Machine is used; the force is recorded as the sample is compressed between the parallel plates. In squeezing flow viscometry there must be a perfect slip between the tested material and

[4] An interesting method based on the oscillation of a capillary was tried (www.vilastic.com). However, due to time constraints the measurements were not concluded.

the plates, which is contrary to conventional shear viscometry. To make sure that the slip-condition is met, lubrication is used. Intact samples can be tested, whereas in shear viscometry with a cup-and-bob measuring geometry the sample is inevitably destroyed as the bob is lowered into the sample. However, it is not possible to vary the strain rate to the same extent; in any case, this is several orders of magnitude lower than that encountered in oral processing. One novelty is the use of Teflon plates instead of lubricated plates, and the introduction of the imperfect squeezing flow setup, in which the lower plate is replaced by a shallow container; this was devised to allow for the testing of semi-solid foods such as stirred yoghurt (Suwonsichon and Peleg, 1999). Results are given as a vector of elongational viscosties or stresses, one for each height measured.

Contraction flow viscometry is another novel method (Stading and Bohlin, 2004). A parabolic nozzle with a defined Hencky strain is used, again in combination with an Instron UTM. Contrary to squeezing flow viscometry only one single elongational viscosity is derived. The effect of shear flow can be taken into account in the calculation of elongational viscosity, but only for Power Law materials for which Power Law parameters can be provided (we did not have these and could not perform this compensation in **Paper IV**). Temperature control is also more straightforward than for squeezing flow viscometry.

However, comparing predictions of sensory viscosities from measured shear and elongational stresses and viscosities (**Paper II** and **Paper IV**), we find no support for the claim that elongational flow is more sensorially important than shear flow, at least in the systems studied here.

5.2.6 Empirical methods

Empirical methods are mostly useful for quality control purposes, correlations between these and sensory data are of limited scientific interest (Peleg, 2006); some extremists even claim that correlations between fundamental rheological parameters and sensory data are irrelevant (Engmann *et al.*, 2006). Empirical methods often outperform fundamental methods in terms of predictive ability. In **Paper II** we have shown that the empirical Posthumus funnel method, modified in such a way that the material leaving the funnel is weighed continously, can yield surprisingly good predictions of sensory viscosity (R^2=0.98), using multivariate prediction models. This is far better than merely determining the efflux time. Interestingly, the flow pattern in the Posthumus funnel is mixed shear/elongation, which may be the reason why it predicts sensory data so well.

5.2.7 Regression models linking sensory and instrumental data.

An assortment of data analytical techniques are available to link sensory and rheological data. This is a core area of sensometrics, the data analytical branch of sensory science. We have used it to treat relationships of the form:

$$Y = aX+b$$

where Y is the dependent variable (normally the sensory data) and X the independent variable (normally the instrumental data). This is simply the linear model, which can be dealt with using different methods. In the simplest case, where the dependent variable Y is merely a vector of one single sensory descriptor, and the independent variable is a univariate, instrumental variable, the equation represents a simple, univariate linear regression, which can be solved by the method of least squares.

The first dairy application of multivariate data analysis appears to be from the 1940s (Harper and Baron, 1948); however, these methods have only become commonplace in the last decade or so. For multivariate X we can use multiple linear regression (MLR), but for computational reasons we normally prefer partial least squares regression (PLSR). Our problem is that the columns in the X matrix are often close to being linearly dependent, leading the equation to be ill-conditioned. For instance, if we regress a matrix of flow curve data on a sensory Y using MLR, we will find the the individual points of the flow curves to be highly correlated (otherwise the flow curve would appear jagged and chaotic); consequently, the matrix equation will be ill-conditioned.

PLSR solves that, but at the expense of resorting to latent variables, which may be difficult to interpret. Another advantage of PLSR is that non-linearities can be dealt with, by adding another latent variable if necessary (Martens and Martens, 2001). But PLSR is basically the most liberal linear regression method. Our contribution to this area has been the use of raw data from Posthumus funnel (see 5.2.6 Empirical methods) as well as other rheological methods.

A recent development in psychorheology has been the introduction of multivariate regression techniques, of which PLS regression is particularly suited to deal with the multicollinearity of the rheological data, to produce prediction models directly from raw data, a concept termed Spectral Stress-Strain Analysis by some (Carson *et al*., 2002; Meullenet *et al*., 1999). The strain rates with the highest explanatory power is directly evident from the regression vector; this procedure is more satifactory than merely calculating the correlation coefficients between the instrumental and sensory data at each strain rate. PLS models using raw data have been found to give better predictions of sensory properties than extracted features, *e.g.* from uniaxial compression curves (Thybo and van den Berg, 2002).

The fact that PLS models of *Oral viscosity* largely outperform univariate models seems to indicate that stress values corresponding to a range of strain rates contribute to the sensorially perceived *Oral viscosity*, which runs counter to the notion that one single strain rate value represent the prevalent strain rate in the mouth, but concurs with the dynamic nature of texture perception.

5.2.3 Measurement uncertainty in sensory-instrumental relationships

Another source of information that is normally neglected in sensory-instrumental studies is contained in the replicates of both sensory and instrumental measurents. There is no logical link between these (sensory replicate number 1 does not relate to instrumental replicate number 1, and so forth). In Figure 9 and Figure 10 the relationship between extensional viscosity (a physical property) and tactile resistance (a sensory property) is depicted for two different viscometry methods, squeezing flow and contraction flow (data from **Paper IV**).

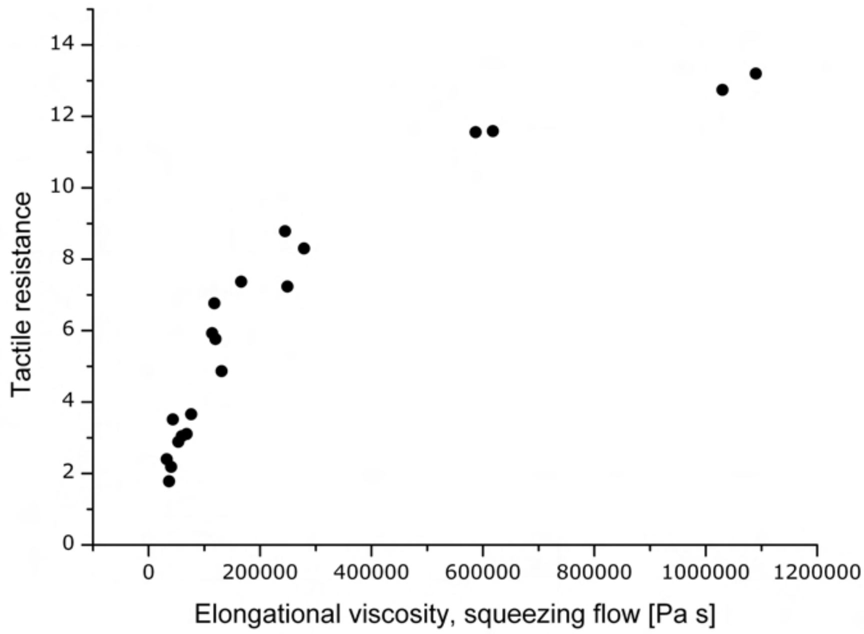

Figure 9. Hand resistance vs. elongational (squeezing flow) viscosity, Trial CC.

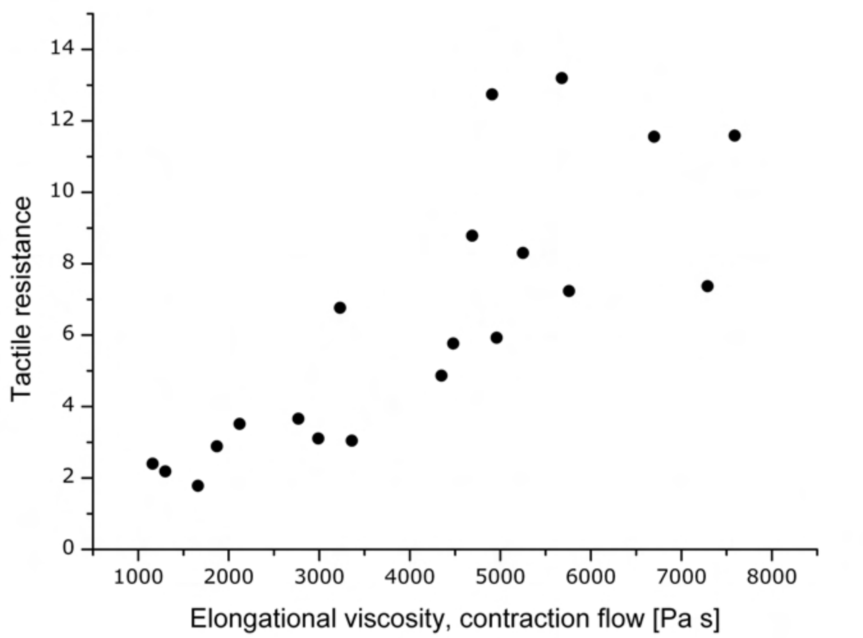

Figure 10. Hand resistance vs. elongational (contraction flow) viscosity.

It should be noted that only averages of replicates can be displayed meaningfully in a scatter plot. Sensory as well as instrumental measurements were performed in triplicate. Introducing replicate as a random factor in a mixed model ANOVA and combining with Measurement Error Methodology has been suggested as a means of separating the true correlation (related to an underlying structure) from error (Brockhoff, 2001). Using this approach we find that the maximal correlation, *i.e.* free from measurement error/uncertainty between viscosity and tactile firmness is 0.98 for squeezing flow 0.97 for contraction flow viscometry, which at first glance may seem surprising, given the level of scatter in the respective plots.

Table 5. Maximal and corrected correlations between key descriptors and elongational viscosities.

Descriptor	Maximal correlation		Corrected correlation	
	Squeezing flow viscosity	Contraction flow viscosity	Squeezing flow viscosity	Contraction flow viscosity
Hand resistance	0.98	0.97	0.92	0.84
Oral firmness	0.98	0.97	0.88	0.86
Creaminess	0.99	0.98	-0.80	-0.80

5.2.4 Imaging and image regression

One reason behind the the popularity of rheological methods in food texture research is the ready numerical output. Relating rhelogical and sensory data is thus mostly trivial. Images, by contrast, do not permit this right away. We would like to deal with models of the form: Y=aX+b, where Y is an array of sensory data, and X is an image, or, rather, some relevant properties derived from an image. In other words: 'image regression'. Image analysis is normally preceded by image pre-processing operations including filtering and thresholding/segmentation (Du and Sun, 2004; Gonzalez *et al.*, 2004).

Confocal micrographs of dairy products are largely isotropic and featureless to the naked eye (in contrast to meat and other foods). One way to deal objectively with this type of images is to measure individual features characteristic of the structure elements of the images. Main features are related to size and shape, *e.g.* of aggregated structures in the CLSM images. As previously mentioned, this has been applied to acid milk gels studies with a wiev to relating microstructure to sensory properties (Pereira *et al.*, 2006). Features such as mean cluster size and mean pore size are univariate properties, and as such straight-forward to relate to sensory data.

In image analysis, an entirely different meaning is given to the term 'texture', although a consistent definition has yet to be coined. A somewhat unprecise definition of image texture is: 'a texture is region (...) that can be perceived as being spatially homogeneous in some sense'

(Carstensen, 2002). This definition includes a totally uniform region, which would not normally be said to have any texture. Image texture has important and diverse applications in the food area (Zheng *et al*., 2006), predominantly in computer vision applications, *e.g.* in tenderness classification of meat (Li *et al*., 2001). Texture feature categories comprise statistical, structural, model-based and transform-based textures (Bharati *et al*., 2004). The much-used grey-level co-occurrence matrices (GLCM) belong to the first group, whereas the Angle Measure Technique (AMT; employed in **Paper VI** and **Paper VII**) as well as the Fourier Transform magnitude spectra (FFT; used in **Paper VII**) belong to the transform-based texture methods. The features extracted by these methods are multivariate, and can be related to sensory data by appropriate methods, *e.g.* PLSR.

The Angle Measure Technique is performed on unfolded images, i.e. the 2D image is first converted to a 1D vector (sevaral options are available - the most simple is to place the rows of the image after each other). The AMT algorithm (here in one of its incarnations (Esbensen *et al*., 1996)) now samples the image vector at a large number of positions.

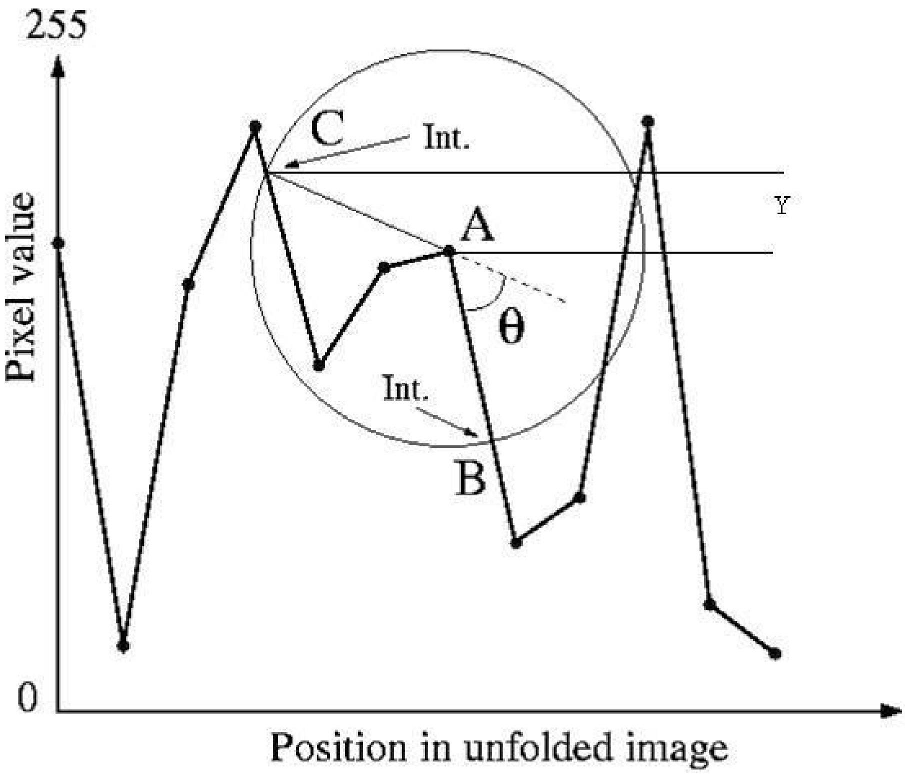

Figure 11. The Angle Measure Technique: computation of angle θ and difference in Y.

With A being the randomly sampled point of the image vector, a circle of radius S with center at A is generated. The points B and C are at the intersection of the image vector with the circle; from this is calculated an angle as well as the projected distances between B and C (Difference X and Difference Y). The angles and differences in Y are then averaged over all the sampled points along the image vector, and the whole process is repeated for several circle radii, S, generating the AMT spectrum in MA and MDY vs. S. The multivariate AMT spectrum characterizes the directional change, expressed as a geometric angle summed in MA, as well as *quasi*-periodic phenomena, expressed in MDY.

The Fourier transform is widely used in image processing and analysis. Applying the Fourier transform to an image, that is, applying the transform on each pixel value row-wise and then column-wise; in practise a 2D Fast Fourier Transform (FFT) algorithm is used. The transformed image is composed om complex numbers; it can be displayed visually by computing its magnitude spectrum, *i.e*. the absolute value of the transform.

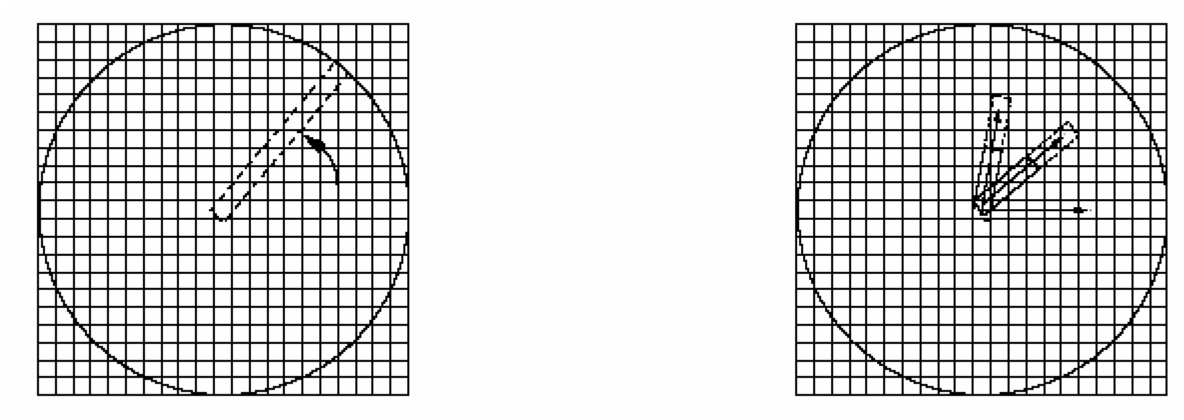

Figure 12. Radial average and angular average of Fourier power spectrum.

The radial average (averaging the polar coordinates) of this magnitude spectrum has been used as a multivariate image feature in this work (**Paper VII**).

Comparisons of the predictive ability of several image features indicate that the Fourier-based and similar feature extraction methods are preferable for images containing periodic phenomena, whereas the AMT spectra seemed to contain more information in the case of more irregular structures (Indahl and Naes, 1998; Kvaal *et al*., 1998).

CLSM images were pre-processed by equalization of local brightness and subsequently submitted to AMT (**Trial Y**) or FFT (**Trial CC**). *Creaminess* was found to be very well predicted in **Trial CC**, which is not surprising given its unequivocal dependence on *Oral graininess*. Predictions of *Creaminess* were poorer in the case of **Trial Y** (R^2=0.34). This could mean that the sensorially relevant, structural variation could neither be captured by CLSM nor by the employed feature extraction procedure. It could also imply that *Creaminess* is not entirely determined by structure in the case of low-fat stirred yoghurt.

Visual texture is an important first sensory cue to several oral descriptors, including creaminess (de Wijk *et al*., 2004; Imran, 1999). By contrast, fluids, as in **Trial AMD**, are largely devoid of surface texture. In **Paper VI** we have described how surface texture can be related to sensory descriptors (**Trial Y** and **Trial CC**) using AMT and PLSR. Using a digital camera, images of plane surfaces of products were captured, and subjected to filtering, elimination of uniform regions and finally feature extraction by AMT. Creaminess could be predicted well for **Trial CC** but not for **Trial Y**, which indicates that creaminess is derived more directly from structure in cream cheese than in yoghurt.

5.2.5 Spectroscopic measurements

Spectroscopic methods, in particular near infrared spectroscopy (NIR), have a long history in the dairy field where they have proven useful in quantitative chemical analysis (Karoui *et al*., 2003). By contrast, the notion of predicting sensory properties from spectroscopic data is quite recent. Again, most work has been done on NIR, *e.g.* on processed cheese (Blazquez *et al*., 2006) and Danbo cheese over the course of ripening (Sorensen and Jepsen, 1998). Fluorescence spectroscopy distinguishes itself by its oustanding sensitivity (100-1000 times more sensitive than other spectroscopic methods). In addition, fluorescent molecules are very sensitive to their environment. Front-face fluorescence spectroscopy, where the surface of the sample is probed, has been used to discriminate sensory and rheological properties in soft cheese (Dufour *et al*., 2001; Kulmyrzaev *et al*., 2005).

Using our cream cheese samples (**Trial CC**), we have evaluated both NIR and fluorescence, as well as low-field nuclear magnetic resonance (NMR) spectroscopy (**Paper IV**). For NIR data, the prediction models are built directly from the raw spectra using PLSR. For fluorescensce spectroscopy data, which are presented in the form of emission-excitation landscapes, PARAFAC (a latent variable decomposition method for higher-order data (Bro, 1997)) was used to decompose the emission-excitation landscapes. The sensory descriptors were subsequently regressed on the resulting PARAFAC scores using multiple linear regression. Reasonable predictions of rheological parameters (**Paper IV**) as well as sensory descriptors (not detailed) were obtained.

The above results are certainly not without interest, but one has to be careful in interpretating them. Because of the proven ability of these spectroscopic methods (in particular NIR) in predicting the concentrations of several chemical constituents of foods, we may have issues of lurking design variables (fat, protein etc.). Whether additional information is present is an important issue; for low-field NMR data on raw potatoes this has indeed been found to be the case (Thygesen *et al*., 2001). For validation purposes it would be interesting to try out several spectroscopic methods on a set of products (or model systems) with a constant chemical composition, but varying processing conditions (homogenization pressure, heat treatment etc.)[5].

[5] We actually did this, but didn't find time to evaluate the results properly.

6 Beyond psychorheology: creaminess

6.1 Modelling creaminess

The difficulty of describing *Creaminess* in purely rheological terms has long been acknowledged (Wood, 1974). A certain level of viscosity combined with a smooth mouthfeel is considered a *sine qua non* condition for obtaining a creamy texture. Several other properties have been claimed to influence *Creaminess*. In concentrated o/w emulsions such as creams, it has been suggested that a high density of evenly sized fat globules contribute to *Smoothness* perception, somewhat along the line of the previously mentioned 'ball-bearing' hypothesis. However, later studies have not been able to demonstrate an effect of oil droplet size on *Creaminess*, *Thickness* or taste (Akhtar *et al*., 2005). Emulsifier type has been shown to influence creaminess of o/w emulsions (Moore *et al*., 1998).

An early attempt at quantitating *Creaminess* is condensed in the formula (Kokini *et al*., 1984):

$$\text{Creaminess} = \text{Thickness}^{0.54}\ \text{Smoothness}^{0.84}$$

This is really beyond the scope of psychorheology, since *Creaminess* is modelled by two sensory variables, namely *Thickness* and *Smoothness*. There is no direct mention of rheological methods, but is is suggested that *Creaminess* can be predicted from rheological and frictional properties, since *Thickness* and *Smoothness* can be predicted from these physical properties. The derivation of this expression is interesting, and says a great deal about the way sensory studies were performed the 1970s and 80s. The first part of the study was to generate vocabularies of texture terms for a series of fluid and semi-solid ranging from apple juice to butter, then eliminate redundant terms and finally use magnitude estimation to quantitate the selected varibles and fit the model. Sensory terms were collated by the untrained panellists individually, as they were told to list as many words as possible which described the texture of the samples. Subsequently the 15 most mentioned words were applied as descriptors in magnitude estimation. In magnitude estimation the panellists are told to score the intensities of a given attribute relative to that of a standard, *i.e.* a ratio scale is used. Averaged attribute scores were then regressed one by one on the remaining descriptors using multiple linear regression, yielding a correlation matrix, from which redundant terms were identified.

As has been pointed out, this approach would not have been used today (Elmore *et al.*, 1999), where descriptive analysis (and the corresponding multivariate data analysis) is considered state of the art (Lawless and Heymann, 1998). In fact, the very validity of magnitude estimation is considered doubtful by some sensory scientists. And, by culling redundant descriptors, we risk bias by the dumping effect.

Table 6. Outline of differences between the sensory work of Kokini et al. and the present work.

	Approach of Kokini et al.	**Contemporary approach**
Sensory methodology	Magnitude estimation	Descriptive analysis
Sensory vocabulary used	Fixed vocabulary previously generated from most used terms mentioned individually by panellists	Vocabulary specific to range of product studied, generated by consensus in panel
Panellists	Untrained panellists	Trained (and paid) panellists
Conditions of test	Room temperature	Temperature in accordance with IDF Standard
Data analysis	Univariate data analysis	Multivariate data analysis

Using a considerably different approach we have found (**Paper II**) for low-fat stirred yoghurt (**Trial Y**):

Creaminess = 0.573 Oral viscosity + 0.650 Smoothness -2.640

The coefficient of determination is only R^2 = 0.42, but this is largely because we have used data from individual assessments, whereas Kokini and Cussler used averaged data. (Using averaged data we obtain R^2 = 0.81).

In **Trial CC** we found *Creaminess* to be largely depending on *Oral graininess*, whereas in **Trial AMD** we found *Creaminess* to be very highly correlated to *Oral viscosity*.

6.2 Sensory basis of creaminess

While the relationship between physical and sensory viscosity is well-established, it is less clear what is behind the concept of *Smoothness*. In engineering terms is makes sense to relate it to friction forces. Sensorily speaking, *Smoothness* can be defined as an absence of *Oral graininess*. At least in some systems, this could imply that *Smoothness* is actually a derived property, with particle properties such as particle size, shape and concentration being the fundamental concept. In fact, neurophysiological studies on macaque monkeys have shown that particle size elicits a response in the brain, as do viscosity stimuli (Rolls *et al*., 2003; Verhagen *et al*., 2004). This could mean that there is an evolutionary advantage in being able to discern *Oral viscosity* and *Oral graininess* in foods, both responses integrating into a *Creaminess* response. From a product technological point of view, *Oral graininess* is more straightforward to operationalize than *Smoothness* (if defined as 'the reciprocal of the frictional force between the tongue and the mouth' (Kokini *et al*., 1977) or as a 'geometrical property' (Peleg, 1983)), in the context of low-fat dairy products. In **Paper V** the effect of fat and protein levels on graininess in low-fat yoghurt has been explored using Response Surface Methodology (RSM).

6.3 A very recent model of creaminess perception in semi-solid foods.

In the Netherlands, de Wijk and co-workers have worked on the subject of *Creaminess* since 1999, mainly using the Dutch vanilla custard product *vla* as a model (de Wijk *et al.*, 2006b). Vla is a semi-solid product, essentially consisting of milk gelled with starch. Fat levels have been varied between 0-15%. Added SiO_2 particles (indeed, not a common food ingredient) in the size range 2-80 µm were found to be detrimental to creaminess[6] (Engelen *et al*., 2005a). Softer polystyrene particles had to be larger to give the same response (Engelen *et al*., 2005b), which could explain why commercial microparticulated whey protein at least are not detrimental to *Creaminess*, despite having particle sizes in the range ~0.1-3.0 µm. Another finding was that product and oral temperature did not affect *Creaminess* ratings, even though the sensory viscosity decreased. The decrease in viscosity was hypothesized to be compensated by other descriptors (Engelen *et al*., 2003). *Creaminess* was found to decrease somewhat with temperature in high-fat custards, and increase a little in low-fat custards. By using noseclips and flavours, the effect olfactory cues and intranasal sensations on creamy mouthfeel was confirmed (Weenen *et al*., 2005).

Based on these findings a qualitative model for *Creaminess* perception was proposed. The model partitions the contributions to creaminess in two: bulk properties (rheological properties of the bolus) and surface properties (lubrication and flavour release provided by fat migrating to the surface of the bolus). The lower creaminess in low-fat custards was thus ascribed to a lack of lubrication, due to the lower fat content (de Wijk *et al*., 2003; de Wijk and Prinz, 2005). Based on PLS models of *Creaminess* as a function of other sensory descriptors, the model was tentatively found to be generalizable to other semi-solids such as mayonnaises, sauces and yoghurts, even if some of the descriptors varied. One could argue that the proposed model disregards the microstucture of the products altogether; in particular the way that fat interacts with other components. In addition, it seems to fail to account for the functionality of fat mimetics such as microparticulated whey protein, unless the lubrication properties of these would be found to match those of fat, as has been suggested by others (Tolstoguzov, 2003). Evanescent wave spectroscopy has been suggested as a method to study deposition-/lubrication phenomena of relevance to *Creaminess* (Malone *et al*., 2003).

[6] Creaminess was evaluated according to a consensual definition: 'Range of sensations typically associated with fat content, such as full and sweet taste, compact, smooth, not rough, not dry, with a velvety (not oily) coating. Food desintegrates at a moderate rate'.

In predicting *Creaminess*, rheological data alone (dynamic oscillation, shear viscometry, critical stress) could only account for at limited amount of information, with cross-validated correlation coefficient Q^2_{CV}=0.48 (Jellema *et al*., 2005); this was deemed reasonably well for high-throughput screening purposes. The idea would be to measure the rheological properties for a large number of samples, and predict *Creaminess* from these. Indeed, it would be interesting to see what the products would look like end after completing several cycles of *Creaminess* optimization using this methodology. Using more ingenious sensory methods (de Wijk *et al*., 2006a), including friction as well as IR reflectance, turbidity and image edge detection on spat out bolus, much better predictions could be achieved (r=0.96 between actual and predicted *Creaminess*), but these methods are hardly useful for high-throughput screening.

7 Conclusions and perspectives

It has been shown convincingly that *Creaminess* in low-fat semi-solid dairy products can be manipulated effectively by both process techological means and by the addition of fat mimetics. We have made the case that *Creaminess*, a key acceptance driver, is largely determined by microstructure along two dimensions: one related to viscosity, another related to particles and graininess. The relative importance of these differs from product to product.

Mastering the fundamentals of the formation and control of *Oral graininess* in low-fat acid milk gel products will enable the dairy industry to develop products with a higher acceptability for the consumer. In the fresh cheese segment in particular there are several process parameters to play around with (pH, salt etc.). Another lead could be to explore the use of pre-concentration of the milk by membrane processes; in concentrated milk, gelation can be induced at a higher pH, which, perhaps in combination with a slightly higher salt level, could be a way to avoid excessive post-aggregation of acid milk gel particles.

With regards to microparticulated protein, there will no doubt be much activity in both fundamental research and more application oriented work in the time to come. There is ample room for developing microparticles with properties (particle size distribution, surface reactivity) tailored to specific applications.

The area of employing image texture methods, to confocal images in particular, is still in need of considerable refinement. One avenue that needs to be followed is that of studying the properties of simulated, isotropic images as models of real confocal micrographs. The ultimate goal would be to use the methods in product R&D. To this end it will be of utmost importance to be able to interpret the loadings in the multivariate image regression models physically, since black box models are of limited use.

In vitro, imitative methods would be of great use to the dairy industry as a means of screening product formulation, but a much higher degree of sophistication than that of the old instrumental TPA method is necessary, both on the hardware side and the data analytical side.

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Multi-way analysis of individual differences in perception of creaminess within a sensory panel

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Abstract

A descriptive analysis of I=25 different plain yoghurts (varying in fat content, protein content and type of added protein) was carried out. A total of J=29 sensory descriptors pertaining to appearance, aroma, flavor, texture and some non-oral manipulation parameters were evaluated by K=12 trained panelists. During the training each panelist was allowed to develop and use their own definitions of the key descriptor *Creaminess*; the remaining 28 descriptors were defined by consensus, and exemplified by reference standards, where feasible. The experiment was carried out in three complete replicates. Data were analyzed by two- and three-way decomposition methods (PARAFAC, unfold-PCA). Subsequently, Creaminess was modeled by two- and three-way PLS and PARAFAC regression with the remaining 28 descriptors as predictors. The results both from bi-linear and multi-linear methods all indicate some degree of individual differences among panelists in their evaluation of Creaminess. Permutation tests, in which individual panelists scores of Creaminess were scrambled randomly with those of other panelists, indicated a high significance of the considered regression models ($\alpha = 10^{-4}$). Furthermore, Creaminess has the highest leverage of the different descriptors; it is clear that it that carries most information about the products. It appears that although the major contribution to creaminess is related to texture and mouth-feel descriptors, a number of flavor descriptors are also involved.

Keywords

Sensory descriptive analysis, Creaminess, Multivariate, Multiway, PARAFAC, N-PLS, PLS

1. Introduction

Within the research field of sensory science 'creaminess' is a highly interesting and much debated topic. It is generally accepted that creaminess has an intrinsic positive hedonic component. It has been demonstrated repeatedly in dairy products that consumers' hedonic response is strongly positively correlated to creaminess. This has been shown to be the case for both strawberry yoghurts (Ward, Koeferli, Schwegler, Schaeppi, & Plemmons, 1999) and plain yoghurts (Folkenberg & Martens, 2003). Furthermore it has been found that consumers' rated perception of creaminess is strongly positively correlated to the same consumers overall liking of the products (Richardson-Harman, Stevens, Walker, Gamble, Miller, Wong, & McPherson, 2000). Thus, naturally there is a high interest in understanding human perception of creaminess.

In some of our previous research on the perception of fat in milk we suggested the use of a so-called meta-descriptor, named 'total fattiness' to describe the overall sensory properties of fat in milk (Frøst, Dijksterhuis, & Martens, 2001). A meta-descriptor in its nature is an overall descriptor that consists of a specific combination of a number of other descriptors[1]. Results from the study of (Frøst et al., 2001) suggest that the use of the meta-descriptor 'total fattiness' is appropriate, as it is the descriptor that alone best preserve the data structure from the full set of descriptors (Dijksterhuis, Byrne, & Frøst, 2002), *i.e.* it is the descriptor carrying the highest amount of information, and best separates the different products under examination. We suggest that creaminess should be considered a meta-descriptor as well.

The scope of the present work is to evaluate a range of three-way and other multivariate data analysis methods for investigating individual differences in perception of creaminess among panelists in a sensory panel. Part of the analysis will be on the individual differences between sensory panelists in their perception of creaminess and the other descriptors as a whole. The purpose is to investigate if the panelists possess the same underlying concept of creaminess, or if there are distinct differences between panelists. The statistical relationship (the correlations) between creaminess and the remaining descriptors will be used to investigate which combination of other sensory properties is important for prediction of creaminess.

Multi-way methods are higher-order generalizations of two-way Principal Component Analysis (PCA) and Partial Least Squares Regression (PLS-R) models. In connection with sensory descriptive analysis, data can be compiled in three-mode array with samples (products) in first mode, sensory descriptors in second mode and panelists in the third mode (Brockhoff, Hirst, & Næs, 1996), as illustrated in figure 1.

[1] Naming the phenomenon a meta-descriptor should be credited to Garmt Dijksterhuis.

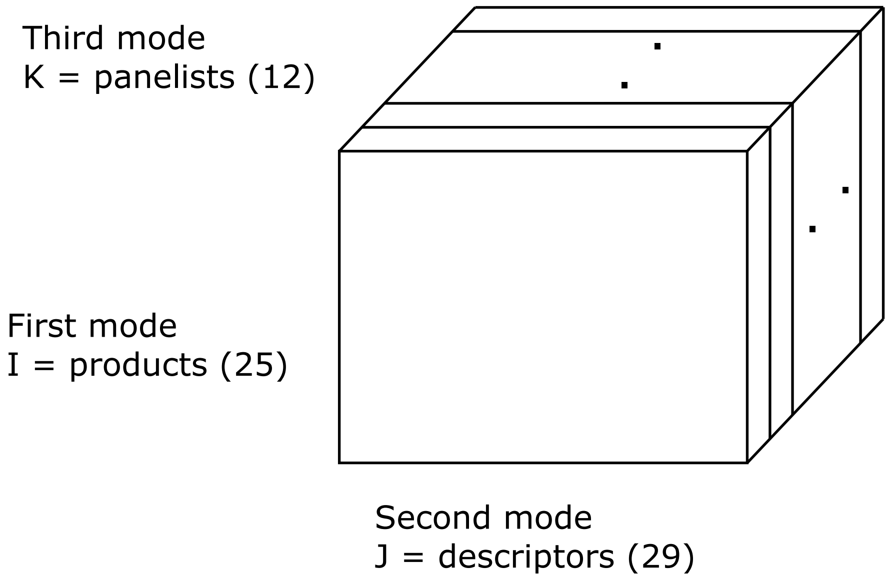

Figure 1: Presently applied and typical arrangement of data array for three-way analysis of data from sensory descriptive analysis.

If the products stem from an experimental design, or other information about the products are available (origin, storage etc.) the array can be extended to higher than three order arrays (Bro, 1996; 1997). In the present work, the differences in sensory properties among individual products, and the overall effects of the experimental design will not be treated thoroughly. This allows us the simplicity of having exactly three-way arrays, and not higher order arrays.

Where two-way methods generally require averaging over one mode, panelists, multi-way methods can be employed to model individual differences between panelists (Bro, 1996; 1997; Brockhoff et al., 1996). Parallel Factor Analysis (PARAFAC, Harshman 1970) or Canonical Decomposition (CANDECOMP, Carroll & Chang 1970) is one such generalization of PCA to higher order arrays. PARAFAC can provide adequate, robust and interpretable models, the most attractive feature being the uniqueness of the solution (Bro, 1997). Similarly, multi-way partial least squares (or N-PLS) regression models is a straight-forward extension of two-way PLS regression to multi-way arrays (Bro, 1996). Equivalent to Principal Component Regression (PCR), the independent variables array *X* can be decomposed using PARAFAC, and the extracted scores regressed on the dependent variable *Y* (PARAFAC regression). As an alternative to three-way methods, the descriptive data can be matrixized, or unfolded[2], into individual matrices and submitted to two-way PCA or PLS-R, which is routinely performed in multivariate software packages like the Unscrambler®. Still, PARAFAC and N-PLS compare favorably to unfolded models, partly because unfolded models can be more difficult to interpret graphically. More importantly, in bilinear unfolding each panelist is presumed to have his own idiosyncratic perceptive model, whereas in the tri-linear PARAFAC model all panelists have a common underlying model, but use it in different proportions. The N-PLS algorithm has been shown to give better predictions than unfold-PLS, the latter being more flexible and consequently more prone to over-fitting (Bro, 1996; 1998).

The issue of preprocessing of data is central to multivariate data analysis. (Bro & Smilde, 2003) have thoroughly discussed the purpose and use of centering and scaling in two-way bi-linear analysis (PCA), but show that they can easily be generalized to higher-order data arrays (PARAFAC). When the data in question stems from sensory descriptive analysis, centering over products (often set as the first mode in three-way arrays) is almost default. Centering the data across the first mode can be seen as a projection onto certain well-defined spaces (the mean in this mode). Often the sensory scientists are not interested in the absolute values of the rating, but rather the variations around the mean, so centering is routinely and almost without exception performed (Brockhoff et al., 1996). Scaling of sensory descriptive analysis data is not strictly necessary, since often all descriptors are assessed with the same scale. In addition, it is assumed and hoped that the panelists use the scale with due reference to the training sessions, i.e. we might surmise that the well-trained panelists inadvertently auto-scale their results. Finally, we could argue that our data should not be scaled to equal variance because of some implied (yet unknown), true difference between descriptor variance, in the set of products under investigation. Still, often the descriptors are different structurally: some are essentially assessments of a perceived intensity, relating to a concentration of some constituents of the sample, *e.g.* "tomato", "lamb", while others probe material properties such as viscosity. There is no such thing as a zero-viscosity yoghurt, but certainly yoghurts devoid of tomato flavor. During the PARAFAC analysis for this particular study we will explore the effects of scaling within the different modes on the models.

While PARAFAC so far has been little applied on data from descriptive analysis, possibly because of not being available in mainstream statistical packages, the algorithmically equivalent INDSCAL procedure from multidimensional scaling has found some use in the context of studying individual differences (Popper & Heymann, 1996), despite requiring a

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[2] Kiers (2000), in an attempt to standardize notation and terminology in multi-way analysis, uses the term matrixization for cutting up multi-way data array into two-way matrices. However, matrixization is more often called undfolding in chemometrics. The term "unfolding" thus has a different meaning here than used in multidimensional scaling, where unfolding is a model for preferential choice (Borg and Groenen, 1997).

transformation of raw scores into a dissimilarity matrix of distances (e.g. Euclidean) between products. In some standard statistical software packages this transformation is done automatically (e.g. SPSS). Chauhan & Harper (1986) and Barcenas, Elortondo, Salmeron, & Albisu (2002) employed INDSCAL on descriptive analysis data, with a particular view to individual differences as well as to comparing the indirect similarity measures derived from descriptive analysis with direct similarity assessments. One disadvantage of the resultant models from INDSCAL is that they are ill-defined with respect to the raw data (Bro, Quannari, & Kiers, 1998). In addition, preprocessing of the raw data, in the form of mean centering and scaling, is largely irrelevant to multidimensional scaling, since only distances are modeled; in a sense, in applying INDSCAL to descriptive analysis data, we are deprived of the option of preprocessing. The differences in results and interpretations of data from sensory descriptive analysis between those two types of algorithms have not been explored much in the literature, and we encourage other scientists to explore this field more.

Many different strategies can be used to assess the magnitude of differences among panelists' ratings of products properties. In the present context the purpose of the study is of a more explorative character, rather than a formal statistical testing situation of a hypothesis. (Dijksterhuis & Heiser, 1995) suggest the use of permutation tests for exploratory multivariate types of data analysis on sensory and consumer data, as a good alternative to more formal tests. We set out to apply one such type of alternative permutation strategy to investigate panelist differences in creaminess perception.

Panelists' individual differences in this study can be of multiple types, it can be differences in rating of Creaminess as such, or it can be differences in the relationships between the other sensory descriptors and the meta-descriptor Creaminess. A way to explore both these types of differences is by unfold-PLS. First the three-way data array is unfolded along the 'slabs' (the third mode – in this case the sensory panelists), and the relationship between the independent unfolded *X*-matrix (sensory descriptors) and the unfolded *Y*-vector (Creaminess) is modeled. Many different types of cross-validation segments can be used, ranging from leaving on sample out at a time to splitting the data into half. The discussion of appropriate cross validation segments is on-going issue, and will always depend on the purpose (confer for instance: Esbensen, Schönkopf, and Midtgaard, 1994; Martens and Næs, 1989; Martens and Martens, 2001). The obvious choice in the context of exploring differences between panelists is to base segmentation on the panelists, such that all data from one panelist is left out for each calibration, and the model is validated against the data from this panelist. This will provide a model that is based on the commonness of the panelists.

The uniqueness of the relationship between the other sensory descriptors of one individual panelist and this panelists' Creaminess rating can be evaluated by subsequently 'scrambling' or block-wise permuting the Creaminess ratings from with each other, panelist-wise. Should any of the permuted models turn out to have predictive ability, in terms of root mean square error of cross-validation (*RMSECV*), equal to or better than the unpermuted model (Baumann, 2003), then the differences in creaminess perception is not significant. Contrary to that, if the *RMSECV* for the permuted results are much higher, it then indicates that the differences are substantial, and creaminess is idiosyncratic.The three steps in analysis of individual differences with unfold-PLS, and illustration of panellist block-wise scrambling of creaminess rating is given in figure 2.

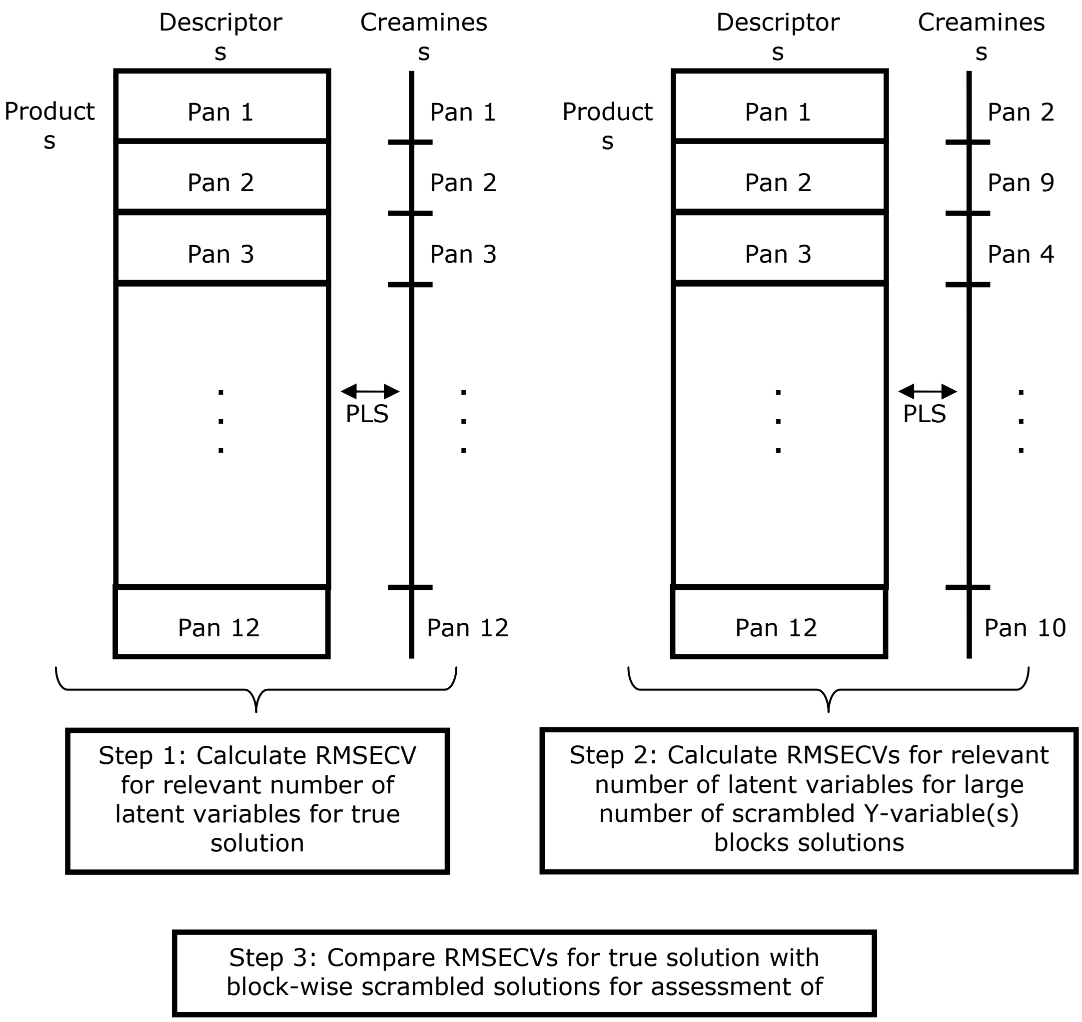


Figure 2: The three steps in analysis of individual differences with unfold-PLS, and illustration of panellist block-wise scrambling of creaminess rating.

2. Materials and Methods

2.1 Products

A total of 25 different types of plain stirred yoghurts were manufactured according to the design briefly listed in Table 1. The table also lists the product abbreviations subsequently used in all plots. In some plots replicates will also be indicated, and will then be specified.
The basic philosophy of the experimental design was to achieve a broad, yet commercially relevant stirred yoghurt sensory property space.

Table 1: The 25 different plain yoghurt types analyzed. Product abbreviations and composition. In plots the first character is a unique code for each product, the second refers to fat level (0, 1, 3), the third refers to protein type (N, L, C, V, M), and the fourth character refers to total protein level (0-4). A fifth character (not shown in this table) refers to sensory replicate (1-3).

Product abbreviations	Fat content (%) (0	1	3)	Type protein added (N S C V M)	Total protein level (w/w%) (0	1	2	3	4)
A-0-N-0	0.3			None (N)	3.3				
B-1-N-0		1.5			3.3				
C-3-N-0			3.5		3.3				
D-0-S-2	0.3			Low protein concentrate - Skim milk powder (S)			4.8		
E-0-S-3	0.3							5.4	
F-1-S-2		1.5					4.8		
G-1-S-3		1.5						5.4	
H-0-C-1	0.3			Commercially available whey protein concentrate (C)		4.2			
1-4-0-C-2*	0.3						4.8		
I-0-C-3	0.3							5.4	
J-1-C-1		1.5				4.2			
K-1-C-2		1.5					4.8		
L-1-C-3		1.5						5.4	
M-0-V-1	0.3			High viscosity producing whey protein concentrate (V)		4.2			
N-0-V-2	0.3						4.8		
O-0-V-3	0.3							5.4	
P-1-V-1		1.5				4.2			
Q-1-V-2		1.5					4.8		
R-1-V-3		1.5						5.4	
S-0-M-2	0.3			Whey protein concentrate with microparticulated whey protein (M)			4.8		
T-0-M-3	0.3							5.4	
U-0-M-4	0.3								6.0
V-1-M-2		1.5					4.8		
X-1-M-3		1.5						5.4	
Y-1-M-4		1.5							6.0

* The product with commercial whey protein concentrate adjusted to 4.8% total protein with 0.3% fat was selected as the reference product to appear in all sensory sessions. Due to data analytical considerations the reference is treated as 4 different products, listed 1-4.

The total fat content was adjusted by addition of full fat cream (40% milk fat) to the milk base. The four different types of protein additions and the levels they were added were chosen in collaboration with Arla Foods Ingredients, Nr. Vium, Denmark, as those that would provide good fat replacers in low-fat and nonfat yoghurts. Simultaneously with the sensory descriptive analysis a number of instrumental measurements (rheological and microstructural characterization) were carried out. This, together with sensory panelist fatigue laid some constraints on the experimental design. On each day of the experiment, seven products were evaluated. To estimate the basic product batch to batch variation one product was selected to be evaluated every day. The yoghurts were produced by Arla Foods Ingredients, Nr. Vium, Denmark in their pilot plant, according to standard methodology for manufacture of stirred yoghurt (blending, prepasteurization, homogenization, pasteurization, cooling, inoculation, incubation, cooling, mixing, filling and final cooling). The same starter culture (YC 183, Chr. Hansen, Denmark) and fermentation conditions (final pH 4.10-4.30) were applied to all products and all replicates. All yoghurts were produced exactly 7 days in advance of sensory evaluation, and kept on storage at 4 °C until the morning of the tests.

The product with commercial protein adjusted to 4.8% total protein with 0.3% fat was selected as the reference products to appear in all sensory sessions. Due to data analytical considerations the reference is treated as 4 different products, listed 1-4.

2.2 Sensory descriptive analysis

Sensory descriptive analysis was performed under normal light with yoghurt (approx. 100 ml) in transparent containers with lids. A panel consisting of 12 external paid panelists was used for the evaluation. All panelists had passed screening tests according to ISO-standards (ISO-8586-1, 1993), and had previous experience with sensory evaluation. Sensory sessions were held in the sensory laboratory at the Royal Veterinary and Agricultural University, which comply with international standards for test rooms (ISO-8589, 1988). In five training sessions panelists were trained on the products, and descriptors were chosen after suggestions from the panel leader on the basis of consensus among the panelists. Each training session had a duration of approximately one and a half hour (In the fifth training session panelists evaluated a subset of the samples for sensory evaluation in the sensory evaluation booths). A total of 29 descriptors were used for the descriptive analysis. Those are listed in Table 2, together with their abbreviations and original Danish terms. For a number of descriptors reference standards were developed, those are listed in table 3.

Table 2: Sensory descriptors, their abbreviations in plots and original words in Danish

Descriptors	Abbreviations in plots	Original terms in Danish
Aroma (Smell)		
Tomato smell	S-Tomato	Lugt af tomat
Lamb smell	S-Lamb	Lugt af lam
Creamy smell	S-Cream	Flødelugt
Buttermilk smell	S-Buttermilk	Kærnemælkslugt
Flour smell	S-Flour	Melet lugt
Visual appearance		
Whiteness	White	Hvid farve
Green	Green	Grøn farve
Grey	Grey	Grå farve
Yellowness	Yellow	Gul farve
Glossy	Glossy	Blankhed
Grainy surface	V-Grainy	Grynethed
Flavour (Retronasal aroma and basic tastes)		
Lamb flavour	F-Lamb	Smag af lam
Butter flavour	F-Butter	Smag af smør
Cream flavour	F-Cream	Smag af fløde
Buttermilk flavour	F-Buttermilk	Smag af kærnemælk
Floury flavour	F-Floury	Melet smag
Sour taste	Sour	Sur smag
Sweet taste	Sweet	Sød smag
Texture and mouthfeel		
Oral viscosity	M-Viscosity	Viskositet
Smoothness	M-Smoothness	Glathed
Melt down rate	M-Meltdown	Nedsmeltning
Astringent sensation	Astringent	Astringerende
Fatty after-mouthfeel	Fatty-AMF	Fedtet eftermundfylde
Dry after-mouthfeel	Dry-AMF	Tør eftermundfylde
Non-oral manipulation		
Non-oral viscosity	NO-viscosity	Gelstivhed
Grainy on lid	Grainy on lid	Grynethed på låg
Viscosity by spoon	Spoon-Viscosity	Viskositet med ske
Continuous flow from spoon	Spoon-Flow	Sammenhængende flydning fra ske
Metadescriptor		
Creaminess	Creaminess	Cremethed

Table 3: Reference standards for sensory descriptors

Descriptors	Abbreviations in plots	Reference material (if any)
Aroma		
Tomato smell	S-Tomato	0.3 L yoghurt (Jersey 0.1% fat, Thise Dairy, Denmark) added 5 drops of Heinz ® Tomato Ketchup
Lamb smell	S-Lamb	See detailed procedure for lamb aroma below*
Creamy smell	S-Cream	Full fat homogenised milk (3.5% fat) and Cream (38% fat) in a 1 to 5 mixture
Buttermilk smell	S-Buttermilk	Organically produced buttermilk (Arla Foods, Denmark)
Flour smell	S-Flour	0.3 L yoghurt (Jersey 0.1% fat, Thise Dairy, Denmark) added 15 mL wheat flour
Flavour		
Lamb flavour	F-Lamb	See above
Butter flavour	F-Butter	Lump of organically produced, salted butter (Lurpak ®, Arla Foods, Denmark).
Cream flavour	F-Cream	See above
Buttermilk flavour	F-Buttermilk	See above
Floury flavour	F-Floury	See above

*Procedure for production of Lamb smell reference: Fry three medium sized lamb chops on medium heat in a skillet. Pour 0.5 L yoghurt (Jersey 0.1% fat, Thise Dairy, Denmark) in a shallow container. Cover the container with aluminium foil and make a reasonable number of small holes. Place the lamb chops on the foil. Wrap close and tight with ceran wrap. Leave overnight in refrigerator at 5 °C.

The use and definitions of the meta-descriptor Creaminess was allowed to be individual for each panelist, as part of the scope of the experiment was to investigate what Creaminess consists of. It would thus be of no use for the experiment to define Creaminess to the panelists. During the first training session, panelists were instructed that they should use their own definitions for Creaminess in their evaluation of the products. Panelists individually wrote down their own short definition of Creaminess after having tasted a number of the samples. Initially in the second training session a summary of the panels' definitions were presented verbally to the panelists. After this the descriptor was not discussed anymore

All samples were kept at 13°C for one hour before sensory sessions. Samples were served only one sample at a time to panelists and were taken out 1-2 minutes before serving. For all evaluation sessions a computerized score collection software (FIZZ, Biosystemes, France) was used. For all descriptors a horizontal 15 cm unstructured line scale was used. For the majority of the descriptors, scales were anchored at the left end with "a little" (in Danish: "lidt") and at the right end with "a lot" or (Danish: "meget"). A few descriptors were anchored differently. M-Viscosity and Viscosity by spoon were anchored with "thin" and "thick (Danish: "tynd" and "tyk" respectively). M-Meltdown was anchored with "slow" and "fast" (Danish: "langsom" and "hurtig" respectively). Sensory analysis of the 24+1 products was carried out in triplicate, and in randomised order within each replicate. In each session only 7 products were evaluated, so a total of 12 sessions were necessary to complete the experiment. Due to a major power failure during the experimental period one of the sessions had to be completely remade, including a new set of yoghurts. This occurred 5 days later than the scheduled last session. The implication of this was that a total of four of the panelists could not participate, resulting in missing data point for these panelists, in that particular session.

2.3 Data analysis

Data analysis was performed in MATLAB Ver. 6.5 (MathWorks, Natick, MA), employing the PLS_Toolbox Ver. 3.0 (Eigenvector Research, Manson, WA) for two-way PCA and PLS-R, and the N-way Toolbox (Andersson & Bro 2000; available from www.models.kvl.dk) for three-way PARAFAC and N-PLS. A number of additional diagnostics for three-way analysis was applied during data analysis, all of them part of the N-way toolbox, or obtainable from the same internet location.

For regression methods (N-PLS and unfold-PLS) data were centered over first mode (products), since the absolute values of intensities are of less relevance for the purpose of this analysis.

A permutation approach was applied for cross-validated unfold-PLSR prediction of Creaminess, to test significance of differences between individual panelists. Initially *RMSECV* (panelists as cross-validation segments) was calculated for a number of latent variables in prediction of Creaminess. Subsequently the panelists' ratings were scrambled randomly and block-wise several times (10,000, out of the 12! possible) so that the ratings from one panelist would substitute those of another, as illustrated in figure 2. Assessment of significance was done by compared the *RMSECV* from the true solution with those of the block-wise scrambled solutions.

3. Results and Discussion

3.1. Decomposition of the sensory data array.

3.1.1 Three-way decomposition (PARAFAC).

After some initial runs we focus on a limited set of models, with different pre-processing options and three to four latent variables (LV's), as indicated in table 4.

Table 4. Overview of different PARAFAC models.

Model #	Pre-processing Centering; Scaling	# Latent Variables	*SSERR*	Iterations	Explained Variance %
1	[0 0 0; 0 0 0]	3	201855	158	84.0
2	[0 0 0; 0 0 0]	4	174773	210	86.1
3	[1 0 0; 0 0 0]	3	127471	36	40.6
4	[1 0 0; 0 0 0]	4	121311	30	43.5
5	[1 0 1; 0 0 0]	3	106891	194	10.6
6	[1 0 1; 0 0 0]	4	103486	70	13.4
7	[1 0 0; 1 0 0]	3	8.519	70	40.5
8	[1 0 0; 1 0 0]	4	8.193	96	42.8
9	[1 0 1; 1 0 1]	3	1.0195	56	10.5
10	[1 0 1; 1 0 1]	4	0.992	39	12.9

In the Pre-processing column, [1 0 1; 1 0 1] denotes mean centering across first and third modes, scaling within first mode, etcetera. SSERR is Error Sum of Squares.

Models #3, #4 and #9, #10 appear to be most stable, i.e. permitting most LV, judging from scree plots, number of iterations and the Core Consistency Index (Bro & Kiers, 2003). Yet, Models #3 and #4 (based on centered data) are preferred to the auto-scaled models #9 and #10, since there might be inherent differences in the variance of descriptors, as argued in the introduction. Centering across first *and* third modes (Models #5 and #6) was tried, and failed miserably (whereas auto-scaling within and centering over first and third modes worked nicely, refer to Models #9 and #10).

Our most coveted descriptor 'creaminess' comes out strongly in the B-loadings plot, both in first and second LV. It is evidently positively correlated to 'Fatty-AMF', 'F-Cream', 'M-Smoothness' and negatively to 'Dry-AMF', 'F-Floury', 'Grainy', among others (figure 3).

The score plot (not shown) reveals a shift from first to second and third replicates (i.e., over time); data from the 1st replicate also seem more spread out. This would mean that panelists, despite being trained on all samples beforehand, spend the first replicate to figure out the samples, and stabilize more in the second and third replicates.

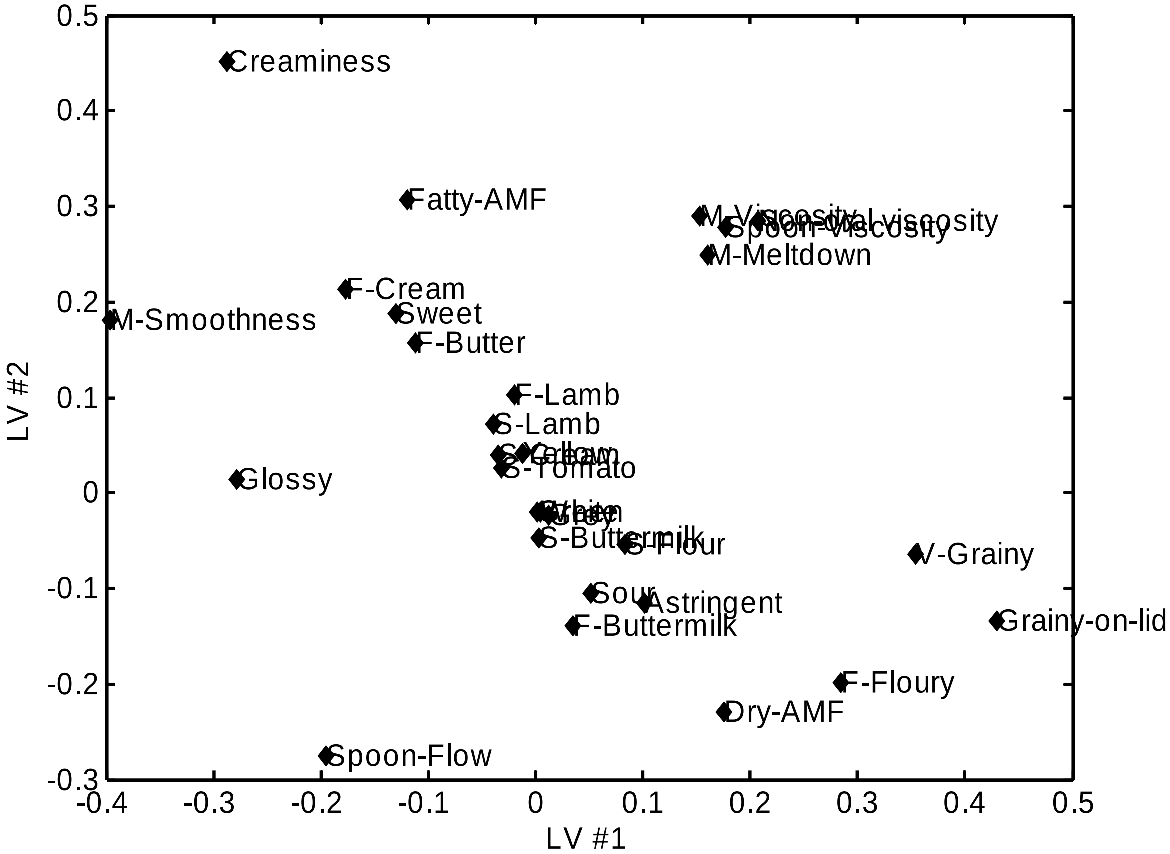


Figure 3: Loadings B-mode (Descriptors) for PARAFAC model

3.1.1.1 Outlier detection

A variety of tools and graphical displays are at our disposal to detect outlying samples: scores, loadings, residuals, leverages, influence plots as well as Resample Influence Plots and Identity Match Plots plots from Jack-knifing (Riu & Bro, 2003) and yet we fail to notice any eye-popping outliers.

3.1.2 Panelist differences

A self-invented addition for displays of outliers is also plotted. We plot Root Mean Square Error (*RMSE*) for each panelist for individual descriptors (figure 4) and summed over all descriptors (figure 5).Panelist 11 have the highest residuals on the descriptor 'creaminess' (not named in figure 4, but it is the descriptor closest to the right side). Still comparing over all descriptors panelist 11 appear no worse than the others (figure 5).

We also notice no apparent structure in residual and leverage plots where panelists (Mode 3) are coded by seniority (1=most experienced, 11 and 12=least experienced). This speaks volumes about the quality of panelist selection and training.

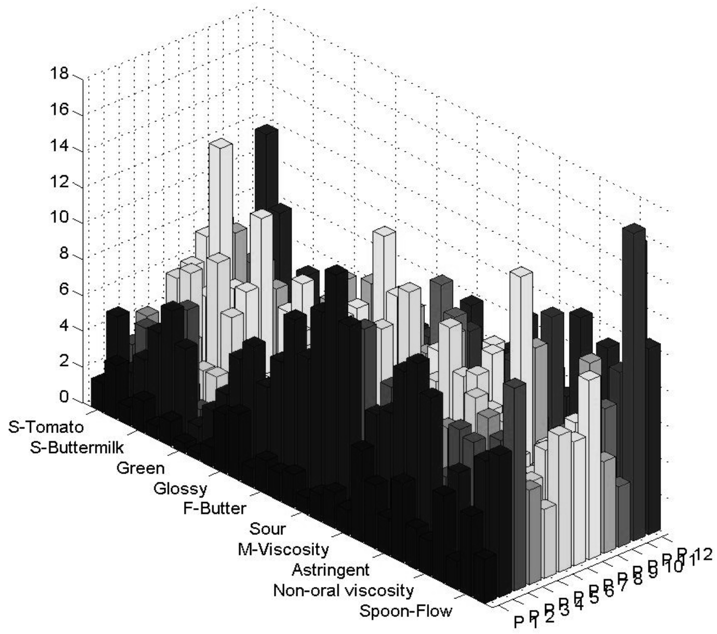


Figure 4: Root Mean Square Error (RMSE) for each panellist for individual descriptors.

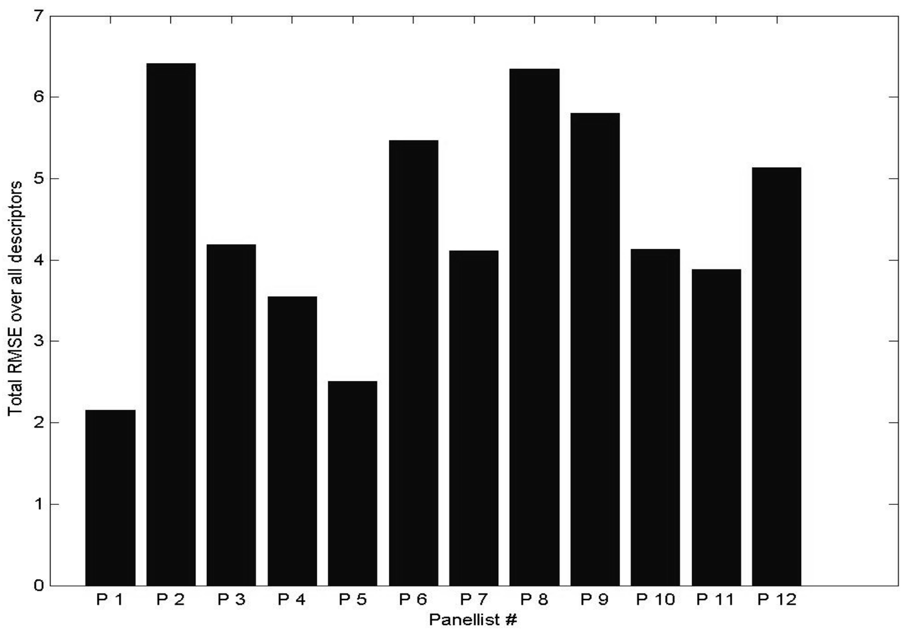


Figure 5: Root Mean Square Error (RMSE) for each panellist summed over descriptors.

As to the optimal number of LV's, Scree plots turn out to be less useful in our case, as we observe no abrupt fall in Error Sum of Squares, (*SSErr*, table 4). Contrarily, the Core Consistency Index (Bro & Kiers, 2003) points unequivocally at a three-factor model. Segmented cross-validation (leaving out multiple, contiguous objects) with 5 segments gives the same result. Furthermore we don't see any new interesting features turning up in score and loadings plot by adding a fourth LV.

We will conclude our PARAFAC modeling here. We should mention that our final model of choice (centered across first mode could possibly be improved upon omission of the relatively unstable protein level 4, or indeed the entire 1st replicate (one third of the samples).

3.1.3. Unfold PCA

Finally a few words about unfold-PCA contrasted to PARAFAC. As mentioned before, unfold-PCA is capable of resolving replicate 1 from the others, just like PARAFAC, but the model complexity is much higher. When the data are unfolded along panelists the loading plot, is quite hopeless, as each descriptor appears 12 times. Alternatively the data can be unfolded along the products, but then each product will appear 12 times. This can be circumvented by using two-way ANOVA-PLSR (cf. Martens & Martens, 2001), but it is no longer an analysis of one data matrix alone.

3. 2 Regression models for prediction of Creaminess (N-PLS and unfold-PLS).

3.2.1 Three way regression models (N-PLS).

3.2.1.1 Outlier detection

The results from initial PARAFAC analysis showed that it was impossible to pinpoint any one product, descriptor or panelist that had distinct outlier behavior. Thus, no data points were excluded in the analysis.

3.2.1.2 Optimal number of components

Cross validation with sensory replicates (3) as cross validation segments showed that five LV's gave the lowest Root Mean Square Error of Prediction (*RMSEP*). However, when increasing the number of segments to 12, then 9 LV's gives the lowest *RMSEP*, and the first local minimum occurs already at the second LV. It is likely that there might be additional learning and improvement of the panelists' capabilities in the first few sessions (*i.e.* the first replicate). By increasing the number of segments beyond the number of sensory replicates, the model will start to incorporate some of this variance that stems from smaller differences between panelists in their use of the descriptors. Also differences in use of the scale within the individual panelist over the course of the 12 session will then start to be modeled. These differences are of little importance, as there are only negligible *RMSEP*-decreases.

A close inspection of the scores and loadings in *X* from the cross validated N-PLS-model, show that the fourth and fifth LV separate those panelists (1, 4, 8, 11) that missed one session from the others panelists. Also LV 4 and 5 separates the 7 products that were evaluated in this session from the remaining 77 (Figures not shown). This shows that even though the fourth and fifth LV's show a decrease in *RMSEP*, the decrease is closely related to the missing values in the data set. We choose to use only the three first LV's for subsequent analysis of panelists' differences in perception of the sensory properties and relations to the meta-descriptor Creaminess.

3.2.2 Prediction of Creaminess

It is of interest to understand which sensory properties can predict Creaminess in plain yoghurt. This is investigated in a series of plots. Figures 6 (A: LV#1-#2 and B: LV#1-#3) show the distribution of the products with regard to their sensory properties (*X*-scores (T)), i.e. all descriptors, except Creaminess. The effect of the experimental design is systematically reflected in the distribution. Samples without added protein are located on the lower left corner of Figure 6A, and increasing levels of added proteins towards the upper right corner. Generally there are relatively little deviations between replicates, as similar products are closely located. LV#3 mainly describes a difference in the two products (D and E) with added skim milk powder, and the lowest fat level (0.3%) from the other products. Figures 7 (A: LV#1-#2 and B: LV#1-#3) show configuration of the Creaminess of the products. The pattern is a low Creaminess on the left side and higher towards upper right corner, in 7A, and a difference in Creaminess in LV#3, mainly in products D and E. Figures 8 (A: LV#1-#2 and B: LV#1-#3) show the configuration of the sensory descriptors in *X* ($W_{J,}$ second mode loadings), and it becomes apparent that the first LV is mostly related to texture (left to right - low to high viscosity), while the second relates more to mouth-feel and flavours (*e.g.* M-Smoothness and F-Cream, Fatty after-mouth-feel *versus* F-Floury and Dry after-mouth-feel). The third LV is mainly related to differences in the descriptor F-lamb. D & E – both 0.3% fat with added skim milk powder) possess a high intensity of this. Products D and E have a high Creaminess, but also a high intensity of lamb flavor.

Figure 6. Score plot X-scores, mode 1 (Products). A: LV #1 and #2. B: LV #1 and #3. Product abbreviations are listed in table 1. Last character in product abbreviation refers to replicate.

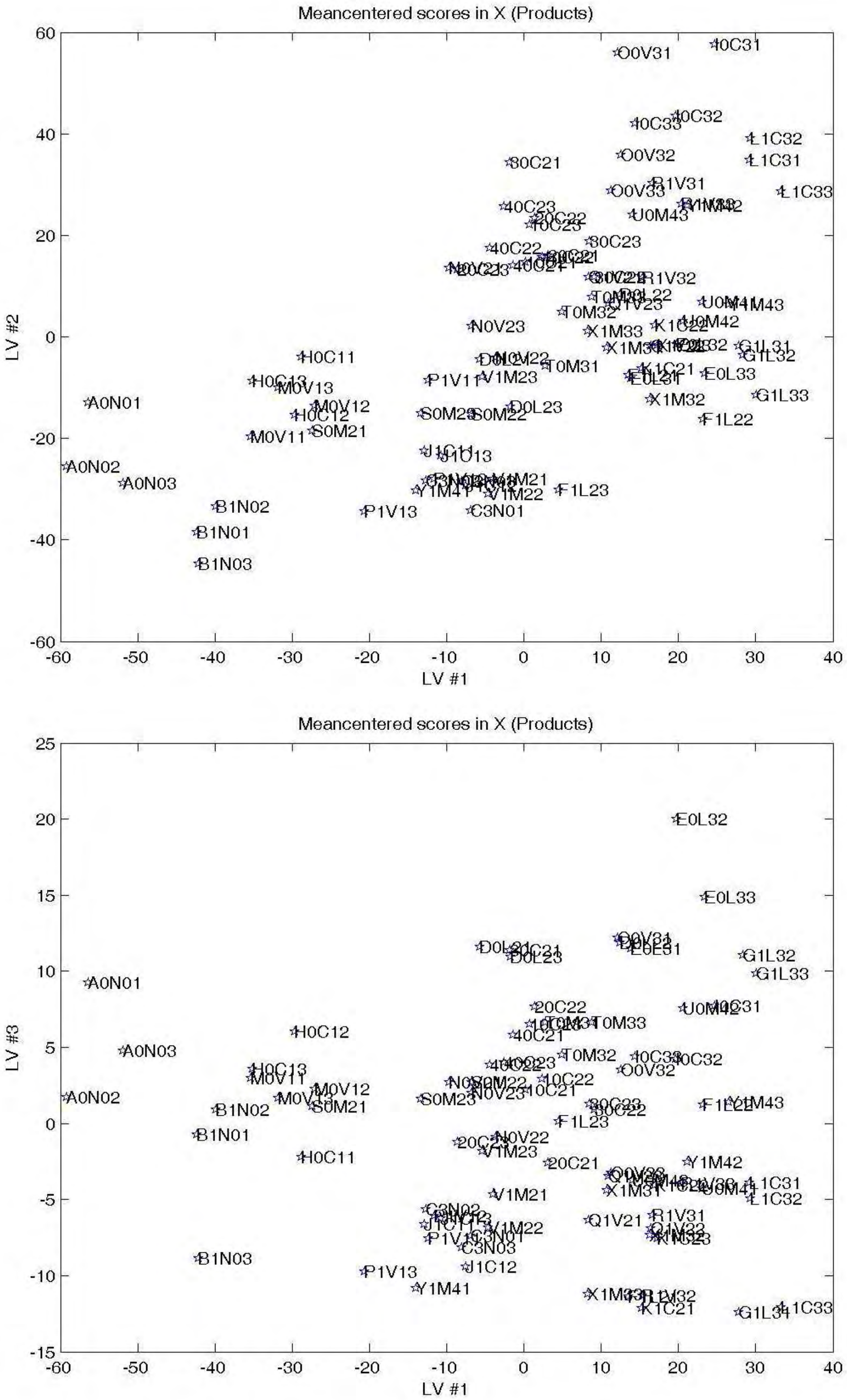
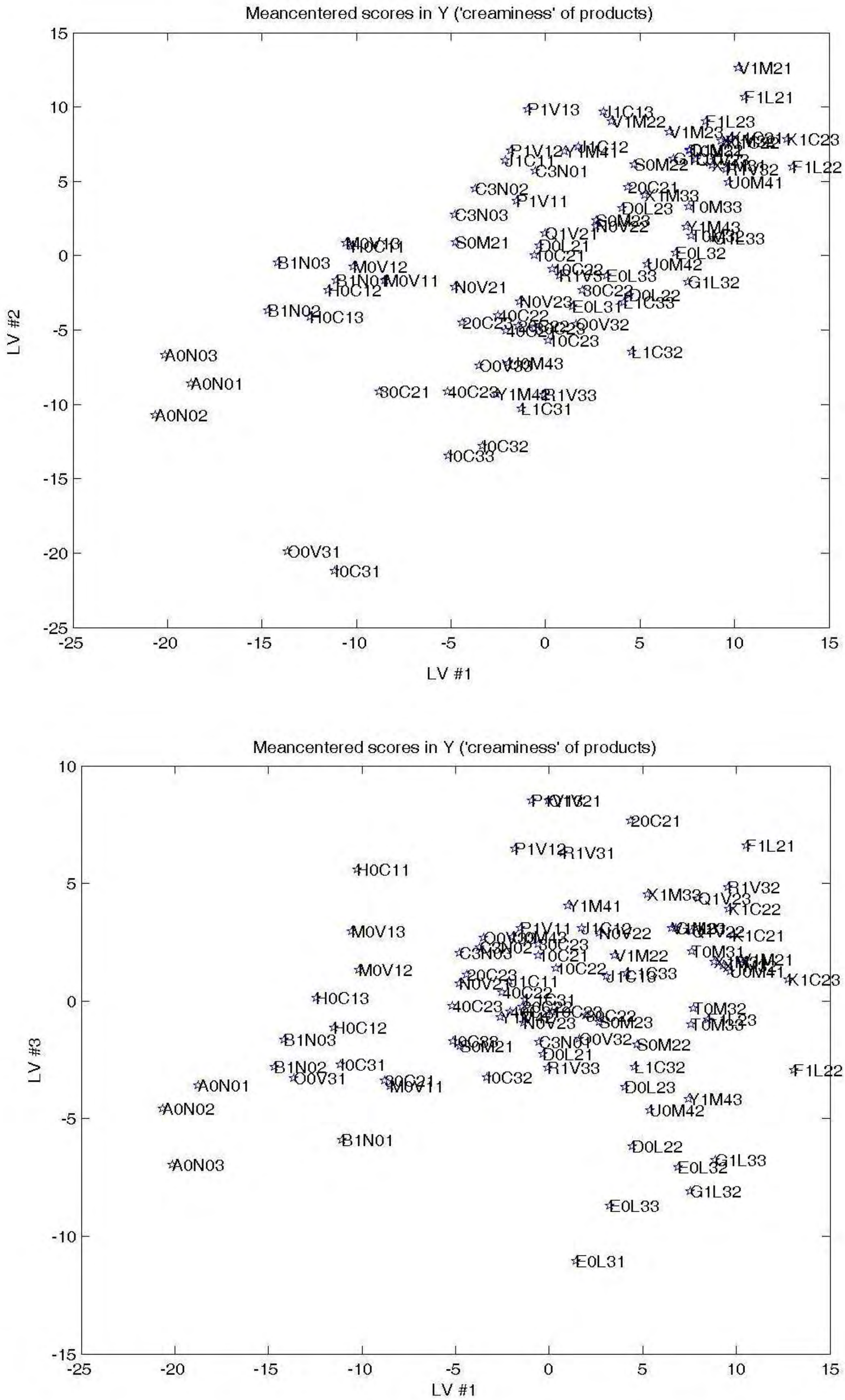


Figure 7: Score plot Y-variables (Creaminess of products). A: LV #1 and #2. B: LV #1 and #3. Product abbreviations are listed in table 1. Last character in product abbreviation refers to replicate.



3.2.3 Panelist differences.

The most interesting part of the analysis is the examination of differences among panelists in their perception of creaminess. Likewise, differences in the configuration of the panelists in their evaluation of the other sensory descriptors and Creaminess are of interest. Figures 9 (A: LV#1-#2 and B: LV#1-#3) show the panelist configuration in the sensory descriptors. Panelists 1 and 6, and to some degree 9, have the highest loadings weights in LV #1, while panelists 3 and 5 have the lowest (figure 9A). Still, absolutely seen the differences are small, as the scale only goes from approx. 0.23 to 0.36. The interpretation of this is that the panelists on the right end (1, 6, and 9) overall give a higher score in the viscosity-related descriptors. And the panelists in the upper part of LV#2 (panelists 2, 3, 8, 9) overall seen give higher scores to the descriptors in that direction (Grainy–on-lid, V-Grainy, F-Floury and Dry-AMF). Figure 9B shows that panelist 9 is the most extreme in LV#3, indicating that the panelist gives high scores in F-lamb, and oppositely, panelists 10 and 12 give very low ratings in F-Lamb. Although it is difficult to know the exact absolute differences, the plots show that panelists 1, 6, and 9 are most different from the averages, and to a lesser extent panelists 2 and 3 are also different in some way.

The panelist configuration in Creaminess is shown in figures 10 (A: LV#1-#2 and B: LV#1-#3). The differences in the first LV are much more apparent for this descriptor alone, the range goes from approx. -0.1 to approx. 0.4. This shows that some panelists (3 and 7 mostly) have loadings weights that are opposed to the others, *i.e.* they score at least of the samples reversed to the other panelists. In LV #2 panelists 2 and 9 show quite different behaviors from the others. In LV #3, panelist 3 has the highest loadings weights, and panelist 12 has the lowest. The configuration of panelists is thus quite different between Creaminess and the remaining sensory descriptors. However, the absolute size of these differences is difficult to assess.

Figure 8: Loading weights plot for X-variables, mode 2 (Descriptors). A: LV #1 and #2. B: LV #1 and #3. Refer to table 2 for full descriptors.

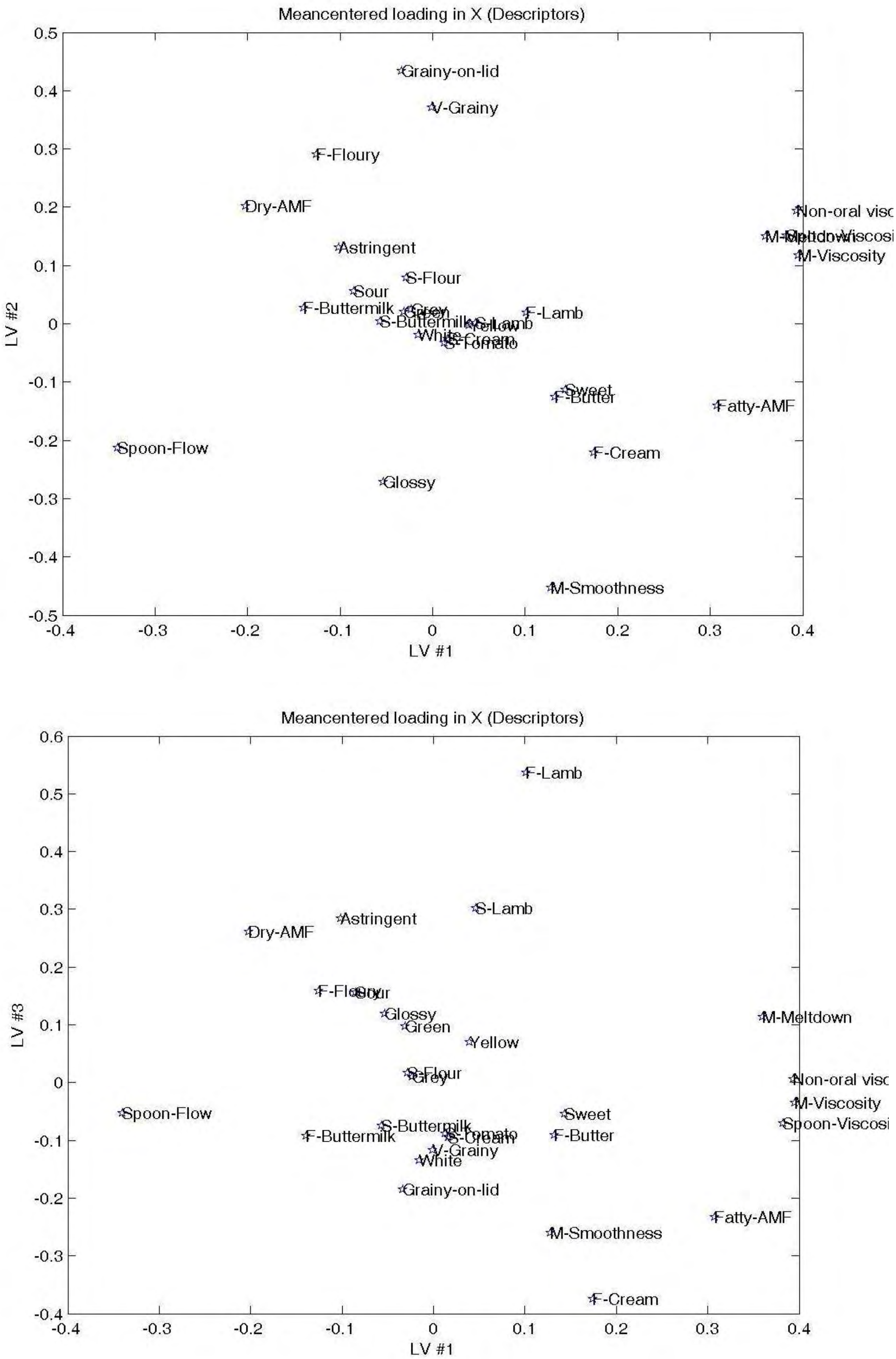


Figure 9: Loading weights plot X-variables, mode 3 (Panellists). A: LV #1 and #2. B: LV #1 and #3.

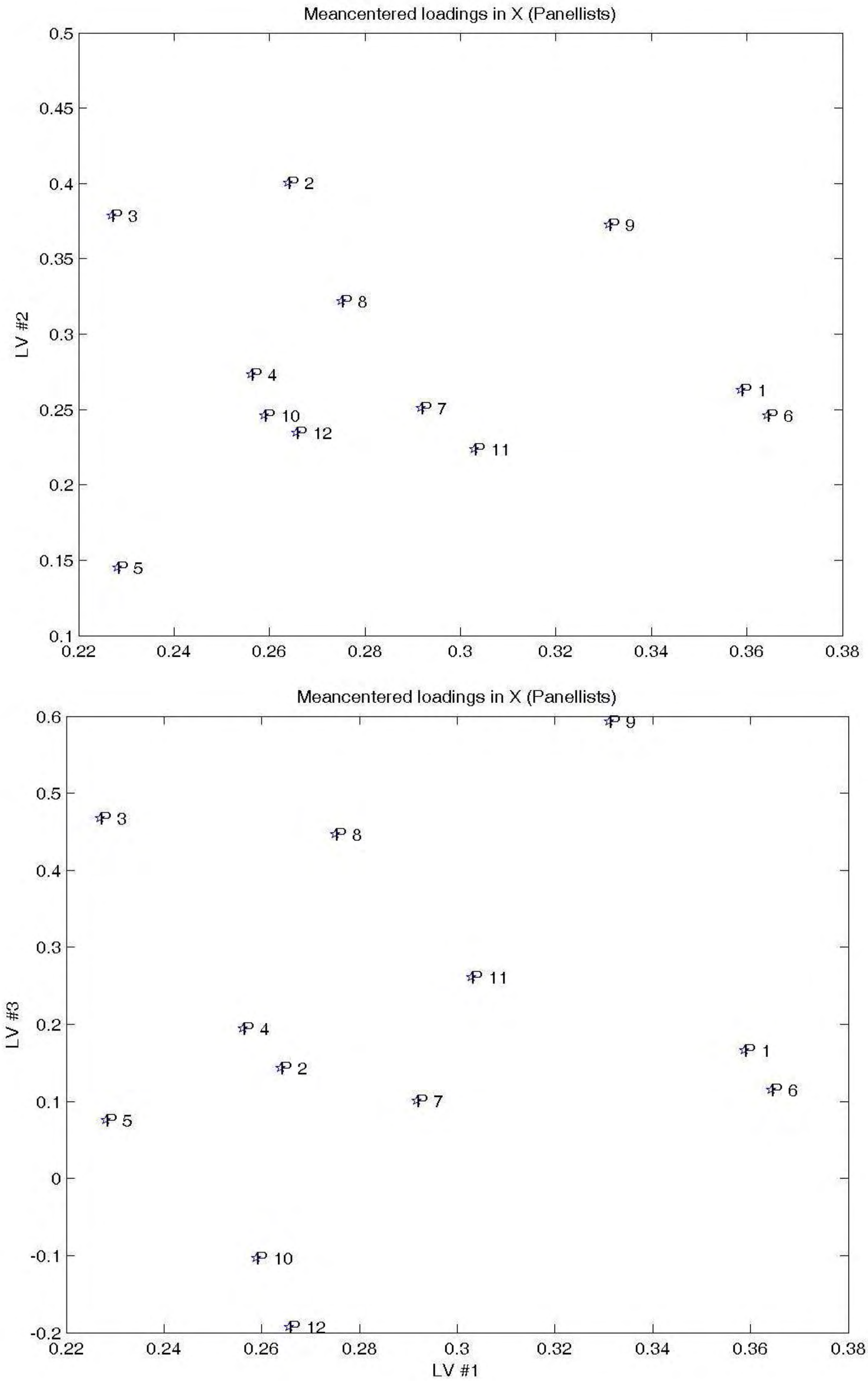
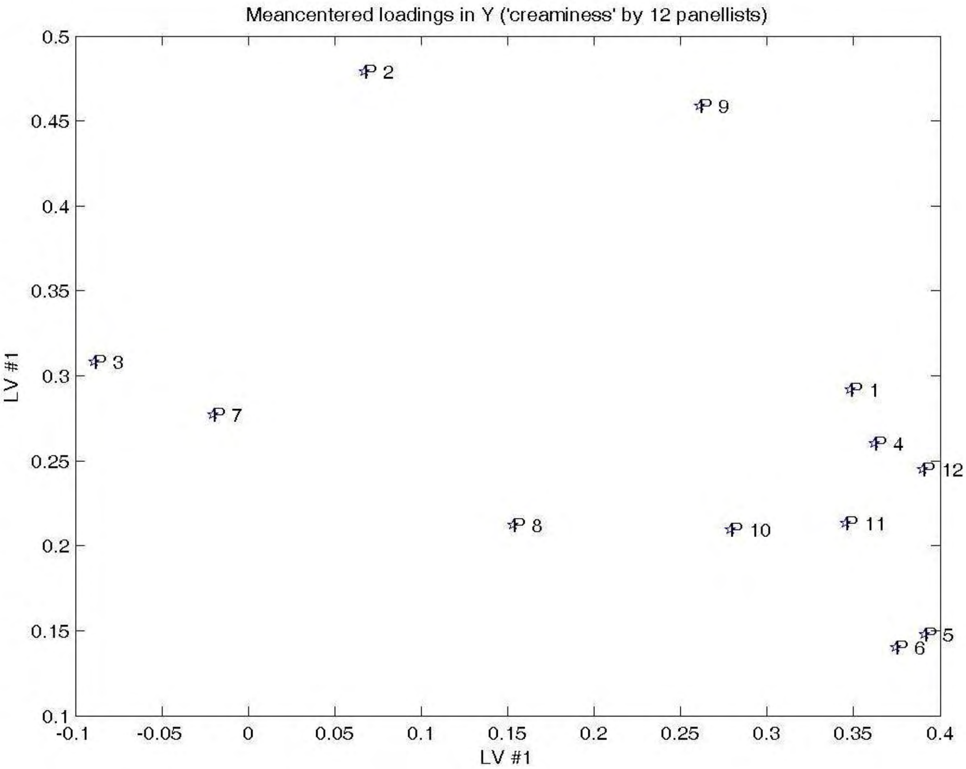
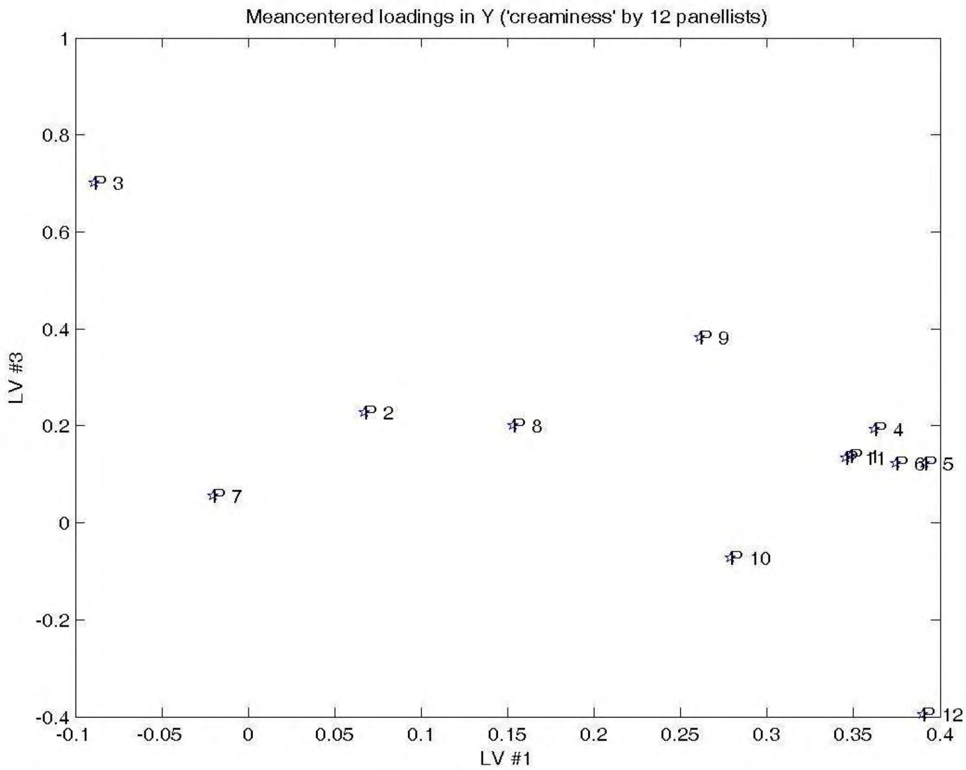


Figure 10: Loading plot Y-variables (Creaminess by 12 panellists). A: LV #1 and #2. B: LV #1 and #3.

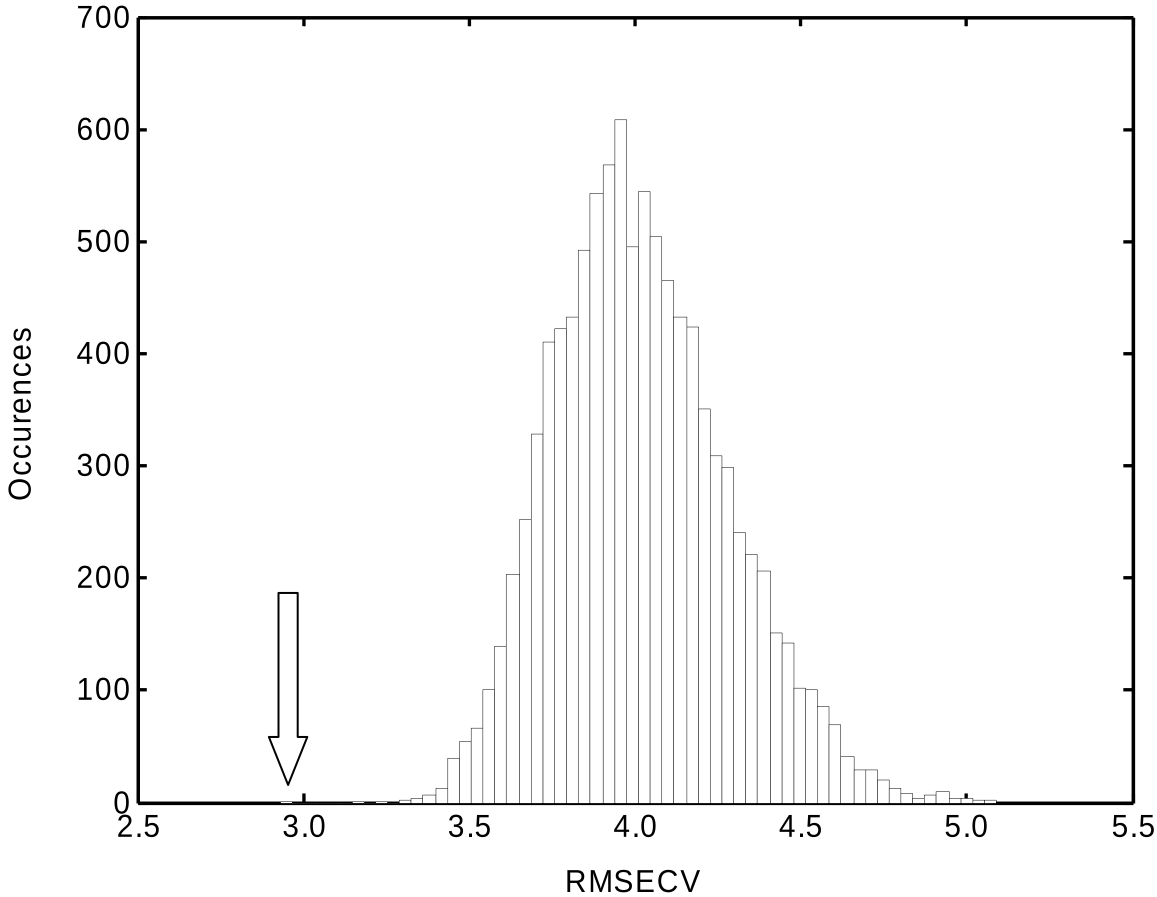




3.2.4 Permutations of unfold-PLS

The outcome of the permutation tests is displayed in figure 11. Since none of the 10,000 permuted PLS models perform even close to the un-permuted model, in terms of *RMSECV*, it is clear that the considered PLS model can be considered relevant with a very high significance ($\alpha = 10^{-4}$). This provides strong support for the notion of creaminess being idiosyncratic.

Figure 11: Histogram from permutation test. Occurences of grouped RMSECV's . Unpermuted model indicated with arrow.



3.2.5 PARAFAC regression.

In PARAFAC regression we first run PARAFAC (3 LV's) on centered *X* (minus Creaminess), and then feed scores and loadings into N-PLS (centered *Y*). Plots from the model (not shown) show that loading weights look similar to those from N-PLS, despite the fact that *Y* has a high variance, and thus is likely to corrupt the decomposition of *X* in N-PLS.

4. Conclusions

4.1 Multi-way models

Using a number of different diagnostic tools it was shown that three latent variables gave robust and reliable models, both in PARAFAC and in N-PLS. Although we attempted to detect outliers by a number of different tools, it was not possible to pinpoint any one sample, descriptor or panelist that had distinct outlier behavior. However, it was clear that the results from the first sensory replicate were somewhat more unstable than those from the remaining replicates.

4.2 Panelist differences

The results both from PARAFAC, N-PLS and unfold-PLS with scrambling permutations all indicate some degree of individual differences among panelists in their evaluation of Creaminess. It is our opinion that the permutation test provides solid evidence that the individual panelists' perception of creaminess differ significantly. The block-wise permutation clearly shows that the statistical relationship between the sensory descriptors and the meta-descriptor is unique for each panelist. However, based on the present experiment and result it can be difficult to conclude exactly what the size of these differences is. Still, as Creaminess has the highest leverage of the different descriptors, it is clear that it is the descriptor that carries most information about the products. In that way the results support that Creaminess is a 'meta-descriptor'.

4.3 Creaminess in plain yoghurts

It appears that although the major contribution to Creaminess is related to texture and mouth-feel descriptors, a number of flavor descriptors are also involved. Based on the broad range of sensory properties of the studied samples, we feel confident in making a general conclusion about creaminess in stirred plain yoghurts. A stirred plain yoghurt with high creaminess is characterized by a relatively high, but not too high, viscosity. It must possess a smooth mouth-feel, and fatty after mouth-feel. The yoghurts with high Creaminess ratings are also high in intensity of fat-related flavors, like cream, and butter, and they are sweeter than those with less Creaminess. A special case is observed in this study, where samples with a clearly perceptible lamb flavor are rated high in Creaminess.

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SENSORY AND RHEOLOGICAL CHARACTERIZATION OF LOW-FAT STIRRED YOGURT

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ABSTRACT

With the specific objective of investigating the sensory concept of creaminess, as well as other sensory attributes obtained from descriptive analysis, a set of 25 samples of stirred low-fat yogurt were submitted to rheological (shear and imperfect squeeze flow viscometry, dynamic oscillation and Posthumus funnel) and sensory testing. Fat levels ranged from 0.3 to 3.5% and protein from 3.4 to 6.0%, and four different protein sources were employed, one being skimmed milk powder, the remaining three were milk protein preparations, one of which contained partially microparticulated whey protein (MPP). Based on averaged data from the sensory panel ($n = 12$*), creaminess could be modeled by two other sensory descriptors, oral viscosity and smoothness (*$R^2 = 0.78$*), but was poorly modeled by the entire set of rheological data. The MPP-containing blend did best in terms of matching the creaminess scores of a control yogurt containing 3.5% fat (no additional protein added).*

KEYWORDS

Creaminess, low fat, viscosity, yogurt, sensory, rheology

INTRODUCTION

The liking of yogurt has been found to be strongly dependent of its fat content (Folkenberg and Martens 2003), and fat replacement with milk protein

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is often the cause of product defects such as syneresis, graininess and off-flavors. Creaminess is thus clearly one of the key attributes in assuring consumer acceptance of fermented low-fat dairy products. Several attempts have been made at addressing the nature and contents of the concept "creaminess." Creaminess can be regarded as a metadescriptor, i.e., a composite of other independent descriptors (the term *metadescriptor* was first coined by Frøst *et al.* 2001, in connection with the sensory perception of fat in milk, where *total fattiness* was used as a metadescriptor). Creaminess has been studied most extensively for oil in water emulsion systems, where it has been primarily related to viscosity, and to a lesser degree the volume fraction of oil (Akhtar *et al.* 2005); the size of the oil droplets has not been found to affect the sensory perception of creaminess significantly. In fluid milk, the total fattiness of a milk sample containing 1.5% fat could be matched by a skimmed milk sample (0.1% fat) to which a thickener, as well as a whitener and an aroma had been added (Frøst *et al.* 2001). This indicates that texture/mouthfeel, flavor and appearance are involved in the perception of fat in fluid milk. Furthermore, in another study, the presence of particles has been found to influence the perceived creaminess of model systems and chocolate mousses (Kilcast and Clegg 2002); the same principle is used in certain fat replacers. Microparticulation of protein results in dispersions of protein particles with a diameter in the range of 0.1–20 μm, capable of emulating the sensory properties of fat (Cheftel and Dumay 1993). In stirred yogurt (a semisolid liquid with weak gel-like properties), both the presence of particles and the additional factors from the fluid milk (appearance, flavor) may be important for the metadescriptor creaminess.

Using magnitude estimation and an untrained sensory panel, creaminess has been modeled as a function of sensory viscosity and smoothness for a wide range of semisolid foods (Kokini and Cussler 1983), with $R^2 = 0.81$.

$$\text{Creaminess} = \text{thickness}^{0.54} \cdot \text{smoothness}^{0.84} \tag{1}$$

The sensory dimensions had previously been reduced to these descriptors by regression analysis. In contemporary sensory analysis, a descriptive analysis by a trained panel would preferentially have been used. Elmore *et al.* (1999) used a trained sensory panel to describe the sensory properties (appearance, texture and flavor) of eight vanilla puddings varying in composition. The sensory properties were subsequently linked to consumers' perception of "liking of creamy texture." In general, Elmore *et al.* (1999) found that the underlying sensations encompassing liking in the vanilla pudding were related to texture, smoothness and dairy flavor. Overall, these observations suggest that the perception of creaminess involves several senses, encompassing at least olfaction, gustation and texture perception.

The cortical representation of food texture, gustatory and olfactory perception shows some degree of convergence in specific areas in the orbitofrontal cortex, where single-neuron recording on primates has shown that some neurons respond to specific patterns of combinations of sensory inputs (Rolls 2004). Interestingly, some populations of neurons in the orbitofrontal cortex in macaque monkeys have been found to respond to viscosity stimuli (carboxymethyl cellulose solutions of different viscosities), while others respond specifically to gritty texture (in the form of suspended microspheres). Some neurons respond unimodally to texture, while others also receive taste input (Rolls *et al.* 2003). The result provides some initial evidence about the information channels that is used to represent the texture and flavor of food. The orbitofrontal cortex is an important region of the brain with respect to representation of the reward value of sensory inputs (Rolls 2004). This indicates that the cortical representation of complex sensory inputs with high reward value, e.g., a food product with high creaminess, may converge in this region.

As a consequence of homogenization of milk for yogurt manufacture, milk fat and casein micelles combine to form pseudocasein particles, and subsequent heat treatment adds denatured whey protein to the structure. Milk fat globules are thus not freely dispersed in yogurt, but rather are embedded within the milk protein matrix. The effect of fat, and fat replacers, is hence indirect, in that it affects the acid milk gel structure. In set yogurt, the addition of microparticulated protein has been found to result in somewhat shorter casein micelle chain structure (and a softer texture), compared to milk fat (Tamime *et al.* 1995).

The Posthumus funnel (Posthumus 1954) is an empirical method commonly used in yogurt production, one of the reasons being its ability to predict sensorially perceived thickness/viscosity (for a Newtonian fluid, the efflux time is proportional to the kinematic viscosity). For instance, Skriver *et al.* (1999) found a correlation of $r = 0.834$ between the efflux time, a measure of apparent viscosity and oral viscosity. Beal *et al.* (1999) and Martin *et al.* (1999) elaborated more on the method, introducing the parameter flowing time coefficient (FTC), defined by

$$\frac{dw}{dt} = -\frac{1}{\text{FTC}}w + k \qquad (2)$$

where w denotes the mass of the material exiting the funnel at time t, and k is a constant. The FTC is a measure of viscosity just as the commonly used efflux time. Unfortunately, the authors did not justify this development in sensory terms, i.e., compare correlation estimates between these two viscosity measures and sensory data; correlations between FTC and oral and spoon viscosity were $r = 0.644$ and 0.290, respectively. The shear rate varies considerably throughout the funnel as the material is drained by gravity. Hellinga *et al.* (1986) modeled the flow in 10 segments of the funnel, and found a decrease in shear rate from 100–500 to 15–90/s during the measurement, although their experimental results did not fit completely to the developed model. It has been noted that a combination of shear and elongational flow is present in the Posthumus funnel (van Vliet 1999, 2002); a similar situation is encountered in the mouth during oral processing.

Few methods are available for measuring elongational properties. The squeezing flow technique ideally yields estimates for biaxial elongational viscosity, and has been adapted to semisolids such as yogurt (Suwonsichon and Peleg 1999; Campanella and Peleg 2002). In the imperfect squeezing flow setup, where the lower plate is replaced by a shallow container, flow conditions are less well defined, and stress values (rather than viscosities) are used as consistency indices; in addition, stresses measured after relaxation for a certain period of time can be used as measures of yield stress. An attractive feature of squeezing flow viscometry is that the material to be tested is not broken down by the measuring system prior to testing, i.e., intact gels such as set yogurt can be tested (Raphaelides and Gioldasi 2005). Contrary to shear viscometry, slip is not a problem, but actually a prerequisite for a proper test.

A recent development in the study of sensory–rheology relationships has been the concept of using raw measurement data as independent variables in multivariate regression models, rather than, say, parameters extracted from flow curves, a soft-modeling approach previously used for modeling sensory data for raw and cooked potatoes from uniaxial compression curves (Thybo and van den Berg 2002), and also termed *spectral stress–strain analysis* (Meullenet *et al.* 1999; Carson *et al.* 2002). In the latter study, a penetrometric method was used to model seven texture descriptors developed from the sensory profiling of yogurt; significant variables were identified by jackknifing. Oral thickness was found to be less accurately predicted ($R = 0.78$) than "spoon impression," visual thickness and slipperiness. The parameters extracted from flow curves (and other forms of raw data) can have more or less of physical and sensory meaning. Skriver *et al.* (1999) preselected shear stresses and moduli based on their correlation to sensory viscosity, and subsequently submitted these, as independent variables (X), along with parameters fitted from empirical flow models, to multivariate modeling of the dependent variable sensory viscosity (Y); in soft modeling, the entire flow curve would be used in the regression model. In viscometry, for instance, shear stresses at different shear rates are likely to be highly collinear; this is a problem in multiple linear regression (MLR), but is handled adequately by latent variable (LV) methods such as principal component regression and partial least square regression (PLSR). These methods are also called for when the number of variables is high compared to the number of samples, which will normally be the case in spectral stress–strain analysis.

In the present work, we have characterized low-fat yogurt sensorially and rheologically, with a particular view to the concept of creaminess. The sensory–rheology relationships are thoroughly investigated to evaluate the predictive value of the different types of rheological measurements on individual sensory properties.

MATERIALS AND METHODS

The experimental design (fat level, protein level, protein type) was primarily chosen to provide a wide sensory space for sensory analysis. Four different milk protein sources were used: skimmed milk powder (SMP), and three milk protein blends (*C*, *M* and *V*), one of which (*M*) contained microparticulated whey protein. The other two were a commercial milk protein blend from Arla Foods Ingredients, Nr. Vium, Denmark (*C*), and a high viscosity-yielding milk protein blend (*V*). Two fat levels were used, 0.3 and 1.5%, as well as a full fat sample containing 3.5% fat (no protein added); there were three protein levels: 4.2, 4.8 and 5.4% (4.8, 5.4 and 6.0 for the M protein type, which required a higher dosage level). Finally, two samples without added protein (fat = 0.3 and 1.5%) were produced. This amounts to a total of $4 \times 3 \times 2 + 3 = 27$ samples. Because of capacity constraints, two combinations were left out of the final design (4.2% protein with SMP, at either fat level), giving a final experimental design of 25 different combinations. A schematic overview of the samples is given in Table 1. These 25 different yogurt samples were produced in triplicate, apart from one reference product which was repeated 12 times (once every sensory session), for a grand total of 84 samples. Yogurts were manufactured in the pilot plant facilities of Arla Foods Ingredients, by blending pasteurized skimmed milk, cream and milk protein, followed by two-stage homogenization at 200/50 bar, 65C, heat treatment at 95C/5 min and inoculation with a high-body, mild-flavor yogurt starter culture (F-DVS YC-183 Yo-Flex; Chr. Hansen A/S, Hørsholm, Denmark) at 42C. Upon reaching pH 4.6, the yogurt was stirred manually and pumped through a strainer and a tubular cooler at a constant back pressure (to ensure a reproducible mechanical treatment), and filled at 22C, before storage at 4C; the final pH was ~4.1–4.3. Because of the inherently unstable nature of stirred yogurt, all sensory and instrumental testing was performed on exactly 7-day-old samples.

The sensory testing comprised a descriptive analysis by a trained panel (12 participants). All panelists were screened according to international standards (ISO-8586-1 1993). Twenty-eight sensory descriptors were developed by consensus during five training sessions of 1.5 h, using refererence samples

TABLE 1.
SCHEMATIC OVERVIEW OF THE YOGURT SAMPLES, ABBREVIATIONS AND COMPOSITION

Product abbreviation	Fat content (%)			Added protein type	Total protein level (w/w %)				
	0	1	3		0	1	2	3	4
0N0	0.3			None (*N*)	3.3				
1N0		1.5			3.3				
3N0			3.5		3.3				
0S2	0.3			Skim milk powder (*S*)			4.8		
0S3	0.3							5.4	
1S2		1.5					4.8		
1S3		1.5						5.4	
0C1	0.3			Commercial milk protein blend (*C*)		4.2			
4 × 0C2*	0.3						4.8		
0C3	0.3							5.4	
1C1		1.5				4.2			
1C2		1.5					4.8		
1C3		1.5						5.4	
0V1	0.3			High viscosity – producing milk protein blend (*V*)		4.2			
0V2	0.3						4.8		
0V3	0.3							5.4	
1V1		1.5				4.2			
1V2		1.5					4.8		
1V3		1.5						5.4	
0M2	0.3			Partially microparticulated milk protein blend (*M*)			4.8		
0M3	0.3							5.4	
0M4	0.3								6.0
1M2		1.5					4.8		
1M3		1.5						5.4	
1M4		1.5							6.0

The different contents of fat (0, 1, 3) and protein (0, 1, 2, 3, 4), and a short description of these proteins (N, S, C, V, M) were added.

* The yogurt with 0.3% fat-added commercial milk protein blend adjusted to 4.8% total protein was selected as a reference sample to appear in all 12 sensory sessions. Because of data analytical considerations, these 12 samples were treated as four different products.

where feasible. Table 2 lists the descriptors, their definitions, abbreviations and original terms in Danish. In addition, the descriptor *creaminess* was evaluated without prior consensus among the panelists, i.e., each panelist used his or her own concept of *creaminess*. The samples were kept at 13C for 1 h prior to the sensory sessions, and served in random order, one sample at a time, and seven samples per session, under normal light conditions in transparent 100-mL containers with lids. The sensory analysis took place in a sensory laboratory complying with international standards for test rooms (ISO-8589

TABLE 2.
SENSORY DESCRIPTORS, THEIR ABBREVIATIONS IN PLOTS AND ORIGINAL TERMS IN DANISH

Descriptor	Abbreviations in plots	Original terms in Danish
Aroma (smell)		
Tomato smell	*S-tomato*	*Lugt af tomat*
Lamb smell	*S-lamb*	*Lugt af lam*
Creamy smell	*S-cream*	*Flødelugt*
Buttermilk smell	*S-buttermilk*	*Kærnemælkslugt*
Flour smell	*S-flour*	*Melet lugt*
Visual appearance		
Whiteness	*White*	*Hvid farve*
Green	*Green*	*Grøn farve*
Gray	*Grey*	*Grå farve*
Yellowness	*Yellow*	*Gul farve*
Glossy	*Glossy*	*Blankhed*
Grainy surface	*V-grainy*	*Grynethed*
Flavor (Retronasal aroma and basic tastes)		
Lamb flavor	*F-lamb*	*Smag af lam*
Butter flavor	*F-butter*	*Smag af smør*
Cream flavor	*F-cream*	*Smag af fløde*
Buttermilk flavor	*F-buttermilk*	*Smag af kærnemælk*
Floury flavor	*F-floury*	*Melet smag*
Sour taste	*Sour*	*Sur smag*
Sweet taste	*Sweet*	*Sød smag*
Texture and mouthfeel		
Oral viscosity	*M-viscosity*	*Viskositet*
Smoothness	*M-smoothness*	*Glathed*
Meltdown rate	*M-meltdown*	*Nedsmeltning*
Astringent sensation	*Astringent*	*Astringerende*
Fatty after mouthfeel	*Fatty-AMF*	*Fedtet eftermundfylde*
Dry after mouthfeel	*Dry-AMF*	*Tør eftermundfylde*
Nonoral manipulation		
Nonoral viscosity	*NO-viscosity*	*Gelstivhed*
Grainy on lid	*Grainy on lid*	*Grynethed på låg*
Viscosity by spoon	*Spoon-viscosity*	*Viskositet med ske*
Continuous flow from spoon	*Spoon-flow*	*Sammenhængende flydning fra ske*
Metadescriptor		
Creaminess	*Creaminess*	*Cremethed*

1988). The samples were scored on a computer screen using a 15-cm unstructured scale; a computerized score collection software (FIZZ; Biosystemes, Couternon, France) was employed. The scales were anchored with "a little" and "a lot" ("*lidt*" and "*meget*," in Danish), except for the viscosity descriptors, for which the terms "thin" and "thick" ("*tynd*" and "*tyk*") were used; *M-meltdown* was anchored with "slow" and "fast" ("*langsom*" and "*hurtig*").

Steady shear viscometry was performed at 13C using a cup-and-bob measuring system (C25; cup diameter: 27.5 mm, bob diameter: 25.0 mm) in a Bohlin VOR – (Malvern Instruments Ltd., Malvern, U.K.) controlled strain rheometer. The initial equilibrium time was 300 s, the constant delay time was 20 s and the measurement time was 10 s. Shear stress was measured at 31 increasing, and subsequently decreasing, shear rates in the range of 0.00919–231/s. The flow curves from viscometry were fitted to the Herschel–Bulkley and QRS (Skriver *et al.* 1993) models using Gauss–Newton nonlinear regression (PROC NLIN in SAS version 8.2; SAS Institute, Cary, NC). In addition, the hysteresis loop area between the up and down flow curves was determined using numerical integration.

Dynamic oscillatory testing comprised strain sweeps (1 Hz) and frequency sweeps (0.005–10 Hz, strain 0.003) in the Bohlin VOR rheometer, again using the C25 measuring system, and an initial equilibrium time of 300 s. From the strain sweep, a yield stress value was derived as

$$\sigma_0 = G'_{\text{yield}}\gamma_{\text{yield}} \tag{3}$$

where G'_{yield} and γ_{yield} are elastic modulus and shear strain, respectively, recorded when the former was reduced to 98% of its maximum value. From frequency sweep data, log(G) versus log(frequency) was plotted for moduli G^*, G' and G''. The slope and intercept of these curves were obtained by linear regression, along with estimates for moduli G at 1 Hz.

Imperfect squeezing flow viscometry was performed using a lower container of diameter $d = 140$ mm, and an upper cylindrical probe ($d = 120$ mm), both made of Teflon (Dupont, Wilmington, DE) and fitted to an Instron UTM model 5564 (Instron Corp., High Wycombe, U.K.) with a 500-N load cell. Samples of 100 mL, corresponding to an initial sample height of 6.50 mm, were poured into the container and allowed to relax at 13C for 30 min prior to testing. The samples were compressed at 0.1 mm/s to a final height of 0.7 mm; stress values obtained during compression every 0.1 mm, at heights 3.0–0.7 mm (where squeezing flow is apparent), were used as consistency indices. Subsequently, the compressed samples were relaxed for a further 180 s to record stresses after 60 and 120 s; these values were used as yield stress estimates.

Posthumus funnels with orifices of 4 and 8 mm were used, and testing was performed at 5C. The material exiting the funnel was collected in a beaker placed on an electronic balance interfaced to a computer. The resulting efflux curve (Fig. 1) was fitted to a second-degree polynomial, yielding the polynomial coefficients a, b and c. In addition, the efflux time was estimated from the efflux curve: $t_{\text{eff}} = t_{280\text{g}}$, i.e., the time it took to collect 280 g of sample from the funnel (this being approximately the mass of yogurt between the upper and lower level marks in the funnel). The FTC was found by plotting mass change

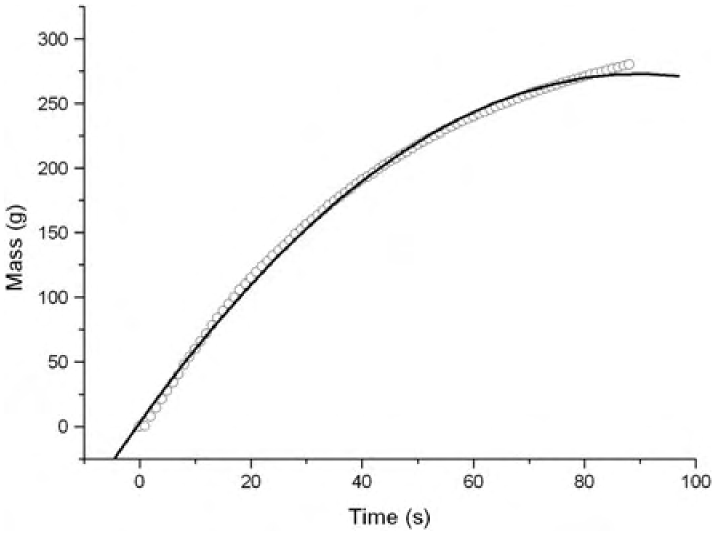

FIG. 1. EFFLUX CURVE FROM POSTHUMUS FUNNEL
The continuous curve represents fitted second-degree polynomial, yielding parameters *a*, *b* and *c*.

per time unit ($\Delta w/\Delta t$) versus mass, and subsequently performed linear regression on the linear portion of the curve. Finally, the mass of residual material in the funnel after emptying (i.e., when $\Delta w/\Delta t = 0$ for at least 5 s) was taken as a potential measure of yield stress.

Data Analysis

Initially, univariate analysis of variance (ANOVA) and multivariate data analysis (ANOVA–PLSR) were applied to analyze the sensory data. Mixed-model ANOVA for individual descriptors was performed with products ($n = 25$) as fixed factors and the panelists ($n = 12$) as random factors. This method is commonly applied for data from descriptive analysis (Næs and Langsrud 1998). ANOVA–PLSR is a multivariate regression method where the effect of design factors on the response variables (here, the sensory descriptors) is evaluated (Martens and Martens 1986, 2001). The method avoids multicollinearity problems by modeling LVs representing the main variation found to be common for the variables. The method evaluates effects of the experimental design variables on sensory properties We have used it here as a graphical alternative to ANOVA (Aastveit and Martens 1986). For multivariate analyses, cross validation was performed, leaving out one replicate at a time (Martens and Næs 1989). Jackknifing with replicates served as the validation tool for all multivariate analyses, comparing the perturbed model parameter estimates from cross validation with the estimates for the full model (Martens and Martens 2000). For multivariate analysis of relationships between consensus sensory descriptors and *creaminess*, the analysis was performed both on the full data set, to explore the effect of differences among the panelists. For other multivariate data analyses, data were averaged over the panelists, and those data were used for the analysis of product properties and relationships with instrumental measurements.

Sensory–instrumental relations were modeled by uni- and multivariate techniques, using R, version 2.0.1 (R Development Core Team 2004); The Unscrambler, version 9.1 (CAMO, Woodbridge, NJ) and MATLAB, version 6.5 (MathWorks, Natick, MA) in combination with the PLS_Toolbox, version 3.0 (Eigenvector Research, Manson, WA). The sensory response variables were submitted to the Box–Cox transformation prior to linear regression modeling; the transformation parameter λ was rounded off to the nearest multiple of 0.50. Apart from the univariate parameters extracted from the three types of flow curves (shear and squeezing flow viscometry; Posthumus funnel), the entire curves (only the up part in case of shear viscometry, and only until the cylindrical part was emptied, i.e., until $m = 280$ g, in the case of the Posthumus funnel, in the remaining places of the data vector the last measured value was duplicated) were used as input for PLSR modeling of *M-viscosity*. The predictive ability of these models, as well as that of univariate linear models of *M-viscosity* regressed on extracted parameters, was validated by leave-one-out cross validation. For the univariate linear models, the predicted values arising from cross validation were retransformed to the original sensory scale prior to computing the root mean square error of cross validation (RMSECV), and were thus directly comparable to those computed for the multivariate models. Regression coefficients, on the other hand, cannot be retransformed and will not be given here.

RESULTS AND DISCUSSION

Sensory Data

The results from ANOVA showed that 26 of the descriptors had significant differences among the samples. The descriptors *S-tomato*, *S-buttermilk* and *Gray* were not found to discriminate significantly between the products, and were thus excluded from further analysis. Figure 2 shows correlation loadings plot of the sensory descriptors from the first two of the three significant LVs (explaining in average 58, 18 and 3% of the variation in sensory data, respectively, data averaged over the panelists). Figure 3 shows distribution and

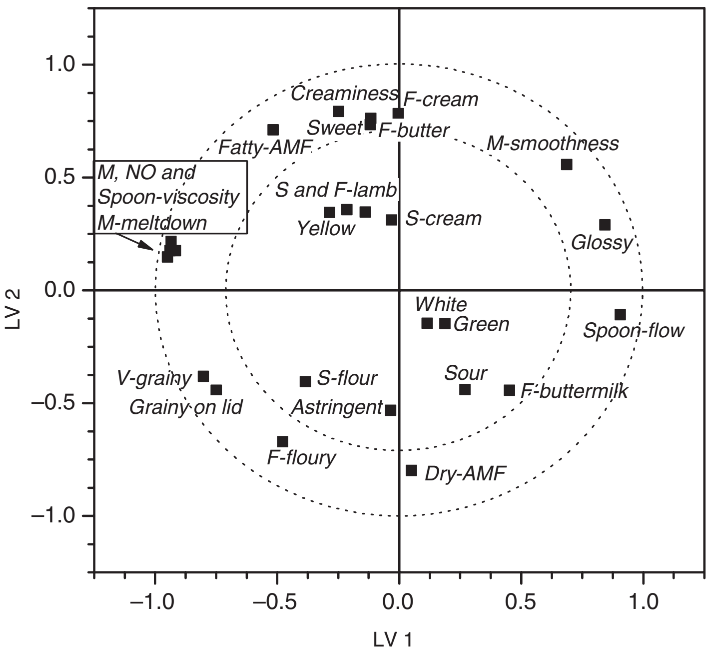

FIG. 2. ANALYSIS OF VARIANCE–PARTIAL LEAST SQUARE REGRESSION CORRELATION LOADINGS PLOT SHOWING HOW SENSORY DESCRIPTORS CORRELATE WITH LATENT VARIABLES (LVs)

The inner and outer circles represent 50 and 100% explained variance, respectively. Refer to Table 1 for descriptor abbreviations.

differences among the products, also indicating the different factors and levels in the object labels. Product differences are referred to along with the explanations in the text.

The grouping and orientation of the descriptors in Fig. 2 show that the first underlying dimension was related mainly to texture. In the left end, a group of descriptors consisting of *M-meltdown*, *NO-viscosity*, *M-viscosity* and *spoon-viscosity* is placed, indicating that products in this direction had a high viscosity and a slower meltdown rate in the mouth. *Meltdown rate* is defined as the rate by which the yogurt bolus breaks down in the mouth. Meltdown rate is the perception of reduction of viscosity as a consequence of manipulation in the mouth and mixing with saliva. Figures 2 and 3 show that the products with a relatively high level of added protein (level 3) had the highest sensory viscosities and the slowest *M-meltdown*. Notice that a further increase in addition of microparticulated protein (M) did not give a further increase in viscosity (Fig. 3). In the opposite end, products with a low score in the

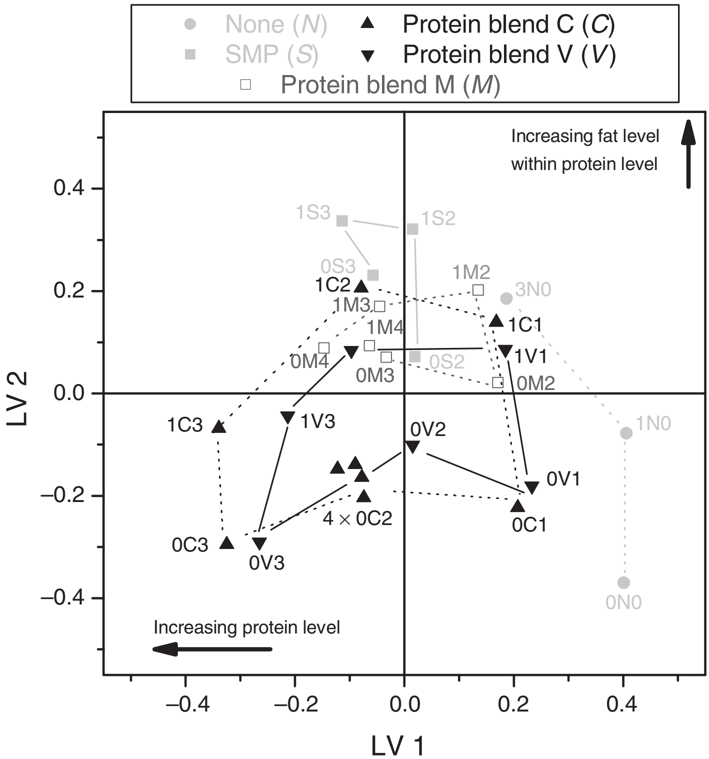

FIG. 3. ANALYSIS OF VARIANCE–PARTIAL LEAST SQUARE REGRESSION SCORE PLOT SHOWING THE DISTRIBUTION OF THE YOGURTS

Sample abbreviation characters refer to fat level (0 = 0.3%, 1 = 1.5%, 3 = 3.5%); protein blend (see top legend); and total protein level (0 = 3.3%, 1 = 4.2%, 2 = 4.8%, 3 = 5.4%, 4 = 6.0%). LV, latent variable; SMP, skimmed milk powder.

aforementioned descriptors are situated. Those products were also high in *glossy* and *spoon-flow* ratings. This is the group of products without or with a low level of added protein (1 and 0 in Fig. 3). To some degree, those products also had a high *smoothness*, particularly the 3.5% fat yogurt ("3NO"), as can be seen as an indication of the fat level in Fig. 3. The second dimension is mainly related to the metadescriptor *creaminess*, located in the top part of the dimension. Products in this direction possessed the highest *creaminess*. A high *creaminess* is correlated with *F-cream*, *sweet* and *F-butter*, and to some degree also to a fatty after mouthfeel (*fatty-AMF*). The products with the highest *creaminess* were "1S2," and others in that direction. Overall, the products with added SMP (labeled S in plots), and the products with level 2 or 3 of added microparticulated milk protein blend (labeled M in plots), had a high *creami-*

TABLE 3.
Creaminess RATINGS FOR ALL INDIVIDUAL YOGURTS (MEAN OF 12 PANELISTS, THREE REPLICATES)

Yogurt sample	*Creaminess* mean rating	Dunnett's one-sided test: P value for sample scoring higher in *creaminess* than control
0N0	1.71	1.0000
1N0	4.12	1.0000
0C3	4.56	1.0000
0C1	5.02	1.0000
0V3	5.07	1.0000
0V1	5.47	1.0000
4 × 0C2	6.99	1.0000
1C3	7.23	0.9990
0V2	7.23	0.9984
3N0	7.86	NA
0S2	8.13	0.8835
0M2	8.13	0.8817
1M4	8.21	0.8476
1V3	8.37	0.7535
0S3	8.38	0.7452
1V1	8.63	0.5542
0M4	8.80	0.4199
1C1	8.98	0.2865
0M3	9.67	0.0256*
1S3	9.70	0.0220*
1V2	9.78	0.0153*
1M3	10.21	0.0057**
1M2	10.49	0.0002***
1C2	11.12	0.0000***
1S2	11.25	0.0000***

Dunnett's pair-wise multiple comparison test against full fat sample "3NO" as control.
NA, not applicable.

ness (see also Table 3). Products in the opposite end of the second dimension were characterized by a low *creaminess*, and this was correlated with a high intensity in *dry-AMF*, *astringent* and *sour*. To some degree, this was also correlated with *F-floury*. Those were mainly products with the lowest fat level (0.3%; 0 in Fig. 3), and added commercial milk protein blend or viscosity-yielding milk protein blend (*C* and *V*, respectively, in Fig. 3). Products in the lower left corner of the plot had a high viscosity (from dimension one), and a relatively low *creaminess*. Lastly, they also had a grainy appearance (*V-grainy* and *grainy on lid*). The third LV (not shown) spanned the difference between samples with and without a high intensity of *F-lamb* and *S-lamb*, and a higher

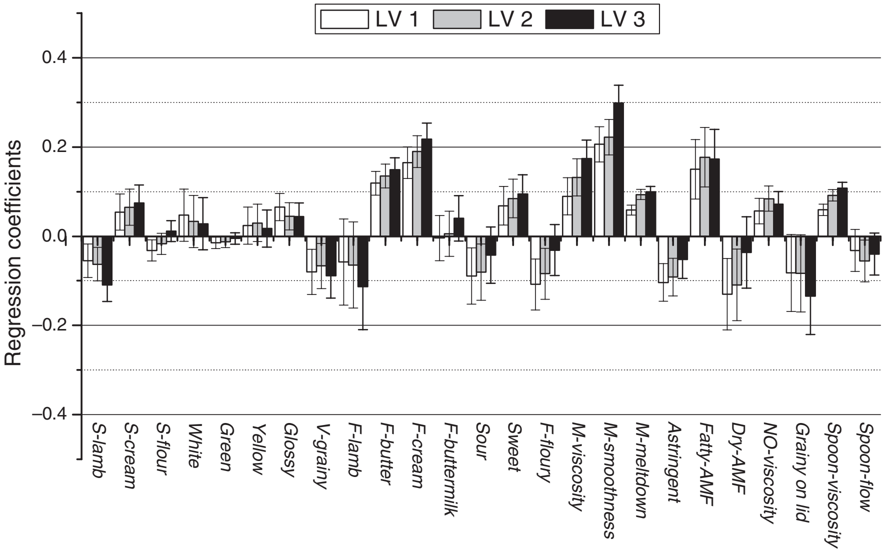

FIG. 4. REGRESSION COEFFICIENTS FOR OPTIMAL NUMBER OF LATENT VARIABLES (LVS) (1–3 LV) FOR PARTIAL LEAST SQUARE MODEL OF CREAMINESS BY REMAINING 25 DESCRIPTORS, MEAN CENTERED
Error bars indicate uncertainty estimates from jackknifing.

astringent rating than what is apparent from LVs 1 and 2. This group of products consisted of all those with added SMP (S). The intensity of *lamb smell* and *lamb flavor* was proportional to the level of added SMP. Furthermore, the effect was most pronounced in the 0.3% fat level. A likely explanation for the occurrence of these descriptors is some off-flavor aroma compounds from the SMP.

Interestingly, *creaminess* was the most discriminating of all 29 sensory descriptors; this has also been seen in other sensory yogurt studies (Muir *et al.* 1997). Further, when regressing *creaminess* ratings (from all the panelists) on the remaining 25 significant descriptors using PLSR, three LVs described the data adequately, capturing 48.8 + 3.6 + 1.2 = 55.6% of the total variance. The analysis showed some differences among the panelists in their rating of *creaminess*. The panelists attributed different weights to flavor versus texture properties (Frøst *et al.* 2004). The relative importance of each consensus descriptor in describing *creaminess* variance is shown by the regression coefficients (Fig. 4). Not unexpectedly, texture-related descriptors carried the most weight, but others, e.g., *S-cream, F-cream*, *F-butter* and *sweet*, were also important. Many of these descriptors were (cross) correlated, and a considerable reduction in the number of explaining variables was possible. In fact, when regressing *creaminess* on just *M-viscosity* and *M-smoothness* (i.e., MLR, excluding interactions), we find with $R^2 = 0.423$ and RMSECV = 2.92.

$$\text{Creaminess} = 0.573 \text{ viscosity} + 0.650 \text{ smoothness} - 2.640 \tag{4}$$

The variance described by those two descriptors alone accounted for as much as 76% of the variance described by 25 descriptors. The two sensory descriptors *M-viscosity* and *M-smoothness* were sufficiently uncorrelated ($r = -0.397$) to ensure that multicollinearity was not a problem (nor was heteroscedacity in this case, so no transformation of the response was necessary).

Table 3 lists *creaminess* ratings for the individual products. From here, it is evident that the addition of all the protein types strongly affected the *creaminess*. Testing the differences in *creaminess* with Dunnett's one-sided test, using the full-fat "3N0" as a control sample, a number of samples had a higher *creaminess* rating. However, among the 0.3% fat samples, only the one with added microparticulated milk protein blend to level 3 ("0M3") was significantly creamier than the full-fat control.

Sensory–Instrumental Relationships

One way to map instrumental and sensory data together graphically is the correlation loadings plot (Fig. 5), based on a partial least square 2 regression model in which *all* instrumental data are employed as the independent variables (*X*), and *all* sensory variables as dependent variables (*Y*). In the correlation loadings plot, the loadings are transformed to (scale–invariant) correlation coefficients between the input variables and the partial least square components (Martens and Martens 2001). The ellipses represent 50 and 100% explained variance. Such a model inevitably has a limited predictive ability, inasmuch as several of the *Y* variables are uncorrelated (Esbensen 2002). Still, from our data, it was evident that several measures of physical and sensory viscosities were located closely to each other in the map, and thus were more or less redundant. This was corroborated by univariate correlations: The correlation coefficient between oral and nonoral viscosity was 0.973 and between oral and spoon viscosity was 0.980. Yet, because of the sequence in which the sensory evaluation took place, it is unlikely that the panelists would remember their different viscosity scores and thus be biased.

Regressing *creaminess* on the entire set of rheological data (using PLSR, with autoscaled data, and two LVs) gave models with modest predictive ability ($R^2 = 0.38$, RMSECV = 1.88). This is expected, as *creaminess* is orthogonally positioned to the sensory viscosity parameters. The proximity of the $a_{4\text{mm}}$ coefficient, in the correlation loadings plot (Fig. 5), hints at a possible correlation to *creaminess*. However, correlations found close to the origin of the correlation loadings plot are most likely spurious.

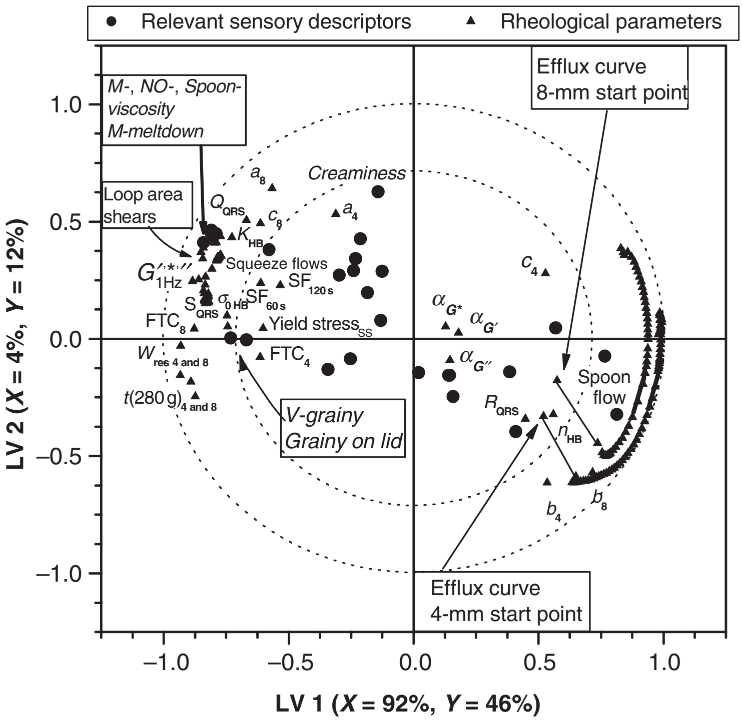

FIG. 5. CORRELATION LOADINGS PLOT DISPLAYING RELATIONSHIPS BETWEEN SENSORY AND RHEOLOGICAL PARAMETERS

Flow curve parameters from shear viscometry (K_{HB}, n_{HB}, Q_{QRS}, R_{QRS}, S_{QRS}); imperfect squeezing flow viscometry (individual stresses and stresses after 60 and 120 s of relaxation, SF_{60s} and SF_{120s}); dynamic moduli (G', G'' and G^* at 1 Hz; slopes of log[modulus] versus log[frequency] plots denoted α located close to the origin are nonsignificant); and Posthumus funnel (the entire efflux curves as well as the extracted parameters a, b and c, the flowing time coefficient [FTC], the efflux time [t] [280 g] and the residual mass [W_{res}]). LV, latent variable.

Modeling of Oral Viscosity (*M-viscosity*)

An assessment of the predictive ability of different rheological parameters should be compared to the inherent uncertainty of the sensory measurements. An ANOVA–PLSR model predicting *M-viscosity* from the design variables (based on the panelists' average ratings), and validated by jackknifing, using replicates as segments, gave an RMSECV of 1.12 ($R^2 = 0.864$). This is the upper limit for precision in a predictive model. Models performing better will have overfitted the data more than the inherent uncertainty of the sensory data permits.

Considering the empirical nature of the method, it is striking that the empirical Posthumus funnel method, in the form of the second-order poly-

TABLE 4.
REGRESSION OF ORAL VISCOSITY (*M-viscosity*) ON RHEOLOGICAL PARAMETERS

	Transformation	R^2	RMSECV
Posthumus funnel			
a_{4mm}	None	0.178	4.91
a_{8mm}	Log	0.884	1.21
b_{4mm}	None	0.505	2.97
b_{8mm}	Square root	0.898	0.97
c_{4mm}	Square	0.194	2.82
c_{8mm}	None	0.507	2.07
FTC_{4mm}	Square	0.302	2.91
FTC_{8mm}	Power 2.5	0.787	1.53
Efflux time, $t_{280g4mm}$	Power 2.5	0.613	2.10
Efflux time, $t_{280g8mm}$	Power 2.5	0.609	2.14
Shear viscometry			
Shear stress at 9.2/s	Power 1.5	0.837	1.12
σ_0 (Herschel–Bulkley)	Square	0.531	2.13
K (Herschel–Bulkley)	None	0.779	1.38
N (Herschel–Bulkley)	None	0.391	2.28
Q (QRS)	Power 1.5	0.694	1.53
R (QRS)	None	0.286	2.48
S (QRS)	Square	0.709	1.60
Imperfect squeezing flow viscometry			
Stress at 1.7 mm	Power 1.5	0.733	1.43
Oscillation			
G^* at 1 Hz	Power 1.5	0.841	1.12
G' at 1 Hz	Power 1.5	0.840	1.12
G'' at 1 Hz	Power 1.5	0.812	1.42
Intercept G^*	None	0.863	1.09

Given are the coefficients of determination (R^2) as well as the root mean square error of cross validation (RMSECV).
FTC, flowing time coefficient.

nomial coefficients *a* and *b*, delivered the best prediction of *M-viscosity* ($R^2 = 0.898$ for the 8-mm orifice) among all of the extracted viscometry parameters (Table 4). This was considerably better than the commonly measured efflux time ($R^2 = 0.556$), or the recently suggested FTC ($R^2 = 0.754$). For all extracted parameters, the 8-mm orifice clearly offered the best predictive ability in terms of RMSECV. Even better was the soft-modeling approach (Table 5); with just two LVs as much as 98% variance was captured for both the 4- and 8-mm orifice. The predictive abilities were roughly the same as for a_{8mm} and b_{8mm}. As is evident from the correlation loadings plot, the first part of the Posthumus efflux curves carried the least amount of information. The first part is closer to the origin of the correlation loadings plot, and thus has less

TABLE 5.
SOFT MODELING OF *M-viscosity* BASED ON POSTHUMUS FUNNEL FLOW CURVE, SHEAR VISCOMETRY AND IMPERFECT SQUEEZING FLOW VISCOMETRY

	Percent variance captured in X (cumulative)	Percent variance captured in Y (cumulative)	RMSECV
Posthumus funnel			
4-mm orifice, LV 1	99.67	82.95	3.68
4-mm orifice, LV 2	99.97	97.69	1.37
8-mm orifice, LV 1	99.93	87.76	3.12
8-mm orifice, LV 2	99.99	98.23	1.21
Shear viscometry			
LV 1	99.30	98.05	1.24
Imperfect squeezing flow viscometry			
LV 1	99.95	97.13	1.51

LV, latent variable; RMSECV, root mean square error of cross validation.

predictive value. Using a subset of samples, for which $t_{280g8mm}$ is less than 60 s, we used an untransformed linear model with $R^2 = 0.756$, RMSECV = 1.73. The efflux time could thus describe the sensorially perceived thickness in a linear fashion, but only over a more limited viscosity range (Fig. 6).

Turning to shear viscometry, we found the best fit to *M-viscosity* at a shear rate of 9.2/s ($R^2 = 0.837$). A better approach to find the sensorially most important shear rate is to inspect the regression vector from the soft modeling of oral viscosity on the shear viscometry flow curve. The regression vector increased monotonously with the shear rate (Fig. 7); the reason why we obtained a different result from the linear regression models based on invidual shear rates is that the Box–Cox transformations (used because of the variance inhomogeneity of *M-viscosity*) used are not optimal for every shear rate considered (a rounded-off value for the transformation parameter λ was used). The soft modeling gave predictions similar to those based on shear stress and the Herschel–Bulkley parameter K. The hysteresis loop area correlated to *M-viscosity* ($R^2 = 0.691$), similar to the $r = 0.867$ found by Skriver *et al.* (1999). The loop area is traditionally regarded as a measure of thixotropy and it was hence inferred that the perception of sensory viscosities not only depended on physical viscosity, but also on the degree of resistance to breakdown. However, this was possibly because the loop area, as is the case for our data, was strongly correlated to shear stress ($r = 0.895$ at a shear rate of 9.2/s). Introducing this shear stress as a partial variable, we only found a partial correlation coefficient of just 0.0232, supporting the notion of a spurious correlation between loop area and oral viscosity.

Stress values from imperfect squeezing flow also correlated to oral viscosity ($R^2 = 0.73$; differences between different heights are not significant),

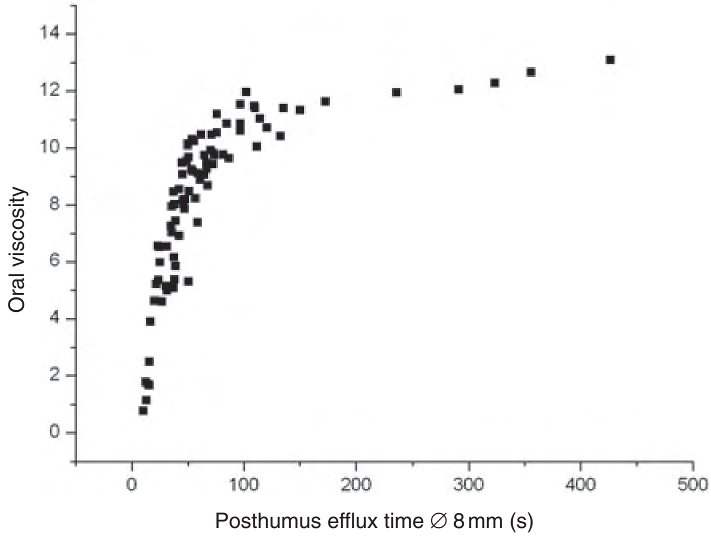

FIG. 6. ORAL VISCOSITY (*M-viscosity*) VERSUS POSTHUMUS EFFLUX TIME, 8-MM ORIFICE.

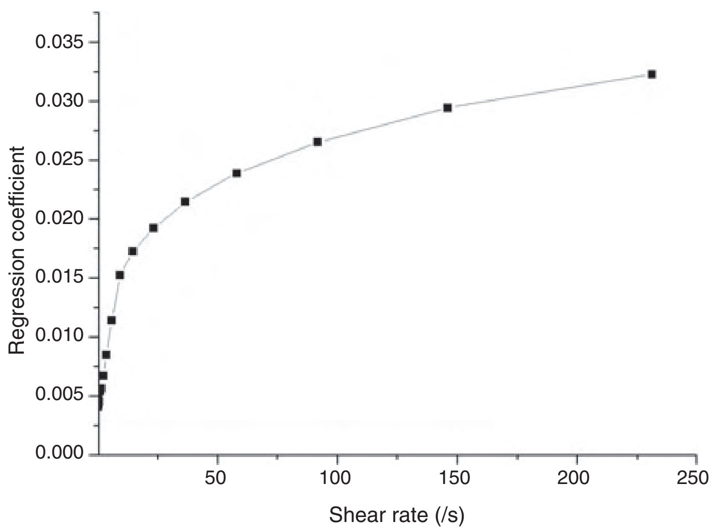

FIG. 7. REGRESSION VECTOR FOR SOFT MODELING OF ORAL VISCOSITY (*M-viscosity*) ON FLOW CURVES FROM SHEAR VISCOMETRY

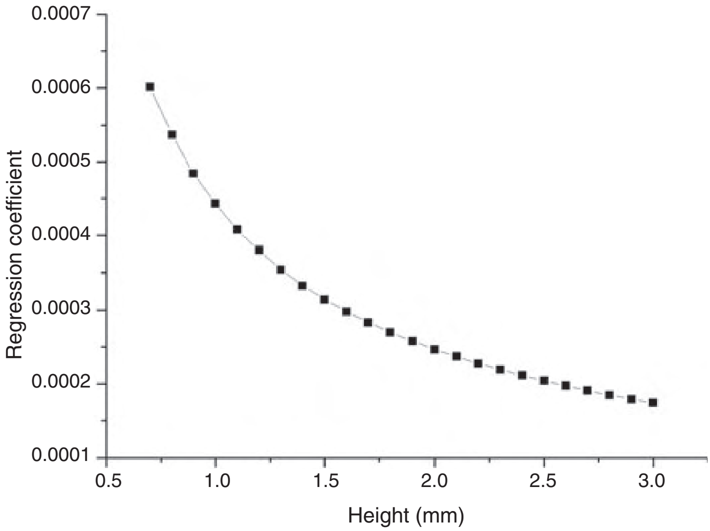

FIG. 8. REGRESSION VECTOR FOR SOFT MODELING OF ORAL VISCOSITY (*M-viscosity*) ON FLOW CURVES FROM IMPERFECT SQUEEZING FLOW VISCOMETRY

despite the fact that the employed strain rate is several orders of magnitude lower than what is encountered in oral processing. Turning to soft modeling, we found the regression coefficient to be decreasing with height (Fig. 8), i.e., the most informative stress value was the one measured at the final height of $H = 0.7$ mm. This is consistent with findings from the discrimination testing on yogurt (Corradini *et al.* 2001); it was found that imperfect squeezing viscometry could resolve two (sensorially indistinguishable) commercial brands of yogurt at heights 0.8 and 1 mm, but not 2 mm. The biaxial strain rate is given by

$$\frac{d\varepsilon_b}{dt} = \frac{V}{2H(t)} \tag{5}$$

where V denotes the displacement rate (Campanella and Peleg 2002). Thus, the smallest height, representing the highest explanatory power relative to oral viscosity, corresponds to the highest strain rate. By and large, in terms of predictive performance, the imperfect squeezing flow method appears to be outperformed by shear viscometry. Squeezing flow viscometry ideally measures elongational viscosity; however, in the imperfect version, where the lower plate is replaced by a shallow container, the flow is less well defined. In the Posthumus funnel, there are both shear and elongational flows, depending on the location; this might be the reason of the difference in predictive performance.

For both shear and imperfect squeezing flow viscometry, one single LV essentially explained the variation in the PLSR models of oral viscosity.

It is noteworthy that dynamic moduli described essentially the same amount of variance of the sensory descriptor *M-viscosity* as does shear viscometry; this was also found by Skriver *et al.* (1999). But again, this could be a spurious relationship because of the strong correlation between shear stress and dynamic moduli. In our case, we found a correlation of $r = 0.878$ between the elastic modulus at 1 Hz and shear stress at 9.2/s. The elastic modulus measured at 1 Hz in the data set varied between 71 and 647 Pa, proof of the considerable texture span encountered in this study.

Yield Stresses

In the correlation loadings plot (Fig. 5), the considered measures of yield stress from shear and imperfect squeezing flow were close to each other and thus correlated, but apparently not to anything of sensory relevance. The same applies to the residual mass of yogurt remaining in the Posthumus funnel after measurement. It is conceivable that other methods for measuring yield stresses, e.g., the vane method, could give more sensorially relevant results.

CONCLUSIONS

The sensory perception of creaminess in low-fat yogurt was clearly dependent on several more sensory variables than just oral viscosity and smoothness, but these were the most important. This corroborates the findings of Kokini and Cussler (1983), even though we have arrived at this conclusion in a very different manner. Because creaminess in low-fat yogurts is not only a result of texture properties, it will be difficult to improve its prediction from rheological properties. As to the sensory relevance of elongational flow, we have not provided conclusive evidence to support the notion that this type of flow is more sensorially important than shear flow.

It was evident that much improved predictions of oral viscosity can be obtained from the Posthumus funnel by recording the mass of yogurt exiting the funnel, either in the form of features extracted from the resulting efflux curve, or by using the efflux curve as an input for soft modeling. In the present study, we have considered a very wide texture span, and the commonly measured efflux time was still effective when predicting the sensory thickness of samples of more equal viscosity. We have demonstrated that a soft modeling approach can result in very precise predictions of textural parameters, even when using an emprical test such as the Posthumus funnel.

ACKNOWLEDGMENTS

This work was supported financially by the Danish Agricultural and Veterinary Research Council (M.B. Frøst) and the Danish Dairy Research Foundation – Danish Dairy Board (T. Janhøj). We thank Arla Foods Ingredients for manufacturing yogurt samples used in this study. The technical staff from the sensory panel, Judith Henning, Maja Nerup Jensen and Lisbeth Pii Nielsen are thanked for assistance with all aspects of sensory descriptive analysis.

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Effect of pre-heat treatment on the functionality of microparticulated whey protein in acid milk gels

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The firmness and water holding capacity (WHC) of acid milk gels with 5.5% protein and 14% total solids, containing varying amounts of skimmed milk powder (SMP), microparticulated whey protein (MWP) and whey protein concentrate (WPC) were investigated. MWP was added before or after pre-heat treatment at 80°C/30 min. On a pure protein basis, MWP contributed the least to both firmness and WHC, whereas SMP contributed the most to firmness, and WPC the most to WHC. Firmness was also found to be slightly higher in gels where MWP had been added after heat treatment whereas WHC was practically identical; it was thus inferred that MWP was not integrated in the protein network, and thus did not contribute to firmness of the gels. For both modelled responses, only minor interaction between the added ingredients was found.

Wirkung einer Vorerhitzung auf die Funktionalität von mikropartikuliertem Molkenprotein in Sauermilchgelen

Die Festigkeit und Wasserbindung von sauren Milchgelen (mit 5.5% Protein und 14% Trockensubstanz) wurde mit unterschiedlichen Zusätzen von Magermilchpulver (SMP), mikropartikuliertem Molkenprotein (MWP) und Molkenproteinkonzentrat (WPC) untersucht. Das MWP wurde vor bzw. nach der Wärmebehandlung (80°C/30 Min.) der Milchgrundlage zugesetzt. Das MWP (als reines Protein berechnet) trug am wenigsten zur Festigkeit bei, das SMP am meisten, während das WPC vorrangig zur Wasserbindung beitrug. Die Festigkeit war kaum niedriger in Milchgelen aus wärmebehandeltem MWP, wohingegen die Wasserbindung praktisch identisch war. Die Ergebnisse implizieren, dass das MWP nicht aktiv in der Strukturbildung der Milchgallerte ist, und somit nicht zur Festigkeit beiträgt. Für sowohl Festigkeit als Wasserbindung erschien nur weinige Wechselwirkung zwischen der Ingredienzen.

21 Milk gels (addition of whey protein, pre-heat treatment) **21 Milchgele** (Molkenproteinzusatz, Vorerhitzung)

1. Introduction

The recent expiry of the Simplesse® patent will probably spark a renewed interest in microparticulated whey protein (MWP). The efficacy of such ingredients in mimicking the sensory perception of fat is well-documented (1), and often related to the particle size; the desirable range is from 0.1 to 2.0 μm (2). Particles below 0.1 μm do not convey an impression of "body", whereas particles above 3.0 μm are sensed as gritty, powdery etc. Alternatively, the observed functionality can be explained in tribological terms, *i.e.* as a lubrication phenomenon, where the rotation of individual microparticles provides the fluidity of a mass of microparticles, which could emulate the surface and rheological properties of fat globules (3).

In an earlier study, we have shown that microparticulated protein is capable of making low-fat (0.3 per cent fat) stirred yoghurt significantly more creamy than a full-fat (3.5% fat) control sample (4). Other studies on soft cheese have shown similar results (5).

On a pure protein basis, addition of MWP has previously been found to result in set yoghurts (3.4 per cent protein, 90% of which from skimmed milk) with a lower firmness than skimmed milk and sodium caseinate, but firmer than with addition of WPC (6). Syneresis (by draining) was on a par with skimmed milk, but clearly lower than in yoghurts made with caseinate and WPC.

In cheese applications, it has been found by transmission electron microscopy that MWP particles, as well as native (*i.e.* non-homogenized) fat globules, are entrapped in the pores of the paracasein network, and consequently do not contribute to the structure formation of the rennet gel (7).

In the particular case of fermented milk products, where a milk base containing protein and other ingredients is heat treated and homogenized (in the original patent covering the application of Simplesse® in set and stirred yoghurt (8), the milk base containing the microparticulated protein was thus homogenized at 17.2/3.45 MPa, and subsequently heat treated at either 90.6°C/5 min, 85°C/30 min or 95°C/6 min), the functionality of the microparticulates is less clear, since the remaining native protein of the MWP, if available at the particle surfaces, could react with the casein, as well as other native proteins, and thus be integrated into the protein network, as is the case with homogenized fat globules. The extent to which they are integrated into the acid milk gel network is of importance, but has so far received little attention in the literature. One study employed transmission electron microscopy (TEM) to visualize the affinity between MWP and milk protein in milk base (heat treated at 90°C/5 min.) as well as after gelation (9). It was found that the whey protein particles interacted less with milk than did recombined fat globules, but interactions took place during gelation. In set yoghurt, casein and MWP particles appeared to be attached to each other in the TEM images, but the yoghurt were softer when made from MWP compared to recombined, anhydrous milk fat. Another study, using scanning electron microscopy (SEM), found that the corpuscular nature of MWP was maintained in the protein matrix (10), causing a more open structure, compared to full-fat and WPC-fortified yoghurts. This was corroborated by a thorough rheological characterization (11) that found the instantaneous compliance J_0 to be significantly higher, and the zero-shear viscosity η_0 to be significantly lower, in yoghurts made from MWP than in full-fat or WPC-fortified yoghurts.

We have thus studied the functional properties of acid milk gels containing micropartulated whey protein, and the effect of heat treatment. Since MWP is commonly combined with micellar casein (in the form of skimmed milk) and native whey protein in fermented milk applications, a mixture design approach was undertaken in order to account for a wide space of protein ingredient combinations.

2. Materials and methods.

The studied MWP was Simplesse® 100E from CP Kelco, Ll. Skensved, Denmark. The protein content (Kjeldahl) was 56.7%, moisture content (after drying at 102°C for 15 h) was 5.12%. Other ingredients in this study were whey protein concentrate LACPRODAN ® 80R (77.5% protein), low-heat skimmed milk powder MILEX 240 LH (37.0% protein) and edible lactose monohydrate VARIOLAC® 992, all from Arla Foods Ingredients, Viby, Denmark. Glucono-δ-lactone (coarse powder, 99%) was from Acros Organics (Geel, Belgium).

The acid milk gels were made by reconstituting the above ingredients in water at 20°C for 2 h, using a magnetic stirrer. Sodium azide was added at 0.02 %. Air was removed by applying vacuum in a capped suction flask for 5 min., using a Heto Master Jet (Heto-Holten A/S, Allerød, Denmark). The reconstituted milk base was subsequently stored at 5°C overnight. Pre-heat treatment of milk bases was performed in a water-bath at 80°C for 30 min, following which the samples were cooled quickly by submersion in water to the acidification temperature (30°C). Glucono-δ-lactone (GDL) was added (*quantum satis* to achieve a final pH 4.60 after 16 h, see below), and the gels were formed in covered 100 mL beakers (50.00 g) for firmness evaluation and centrifuge tubes (30.00 g) for WHC determination.

In the experimental design, all the samples have a base protein content of 3.5% from SMP and, in addition, 2% more from a mixture of SMP, MWP and WPC, defined by an augmented simplex-lattice mixture design (12), see Fig. 1. Total solids was maintained constant at 14 g/100 g, by addition of lactose monohydrate. The mixture design was repeated twice: once with MWP included in the milk base prior to heat treatment, and a second time where the MWP (if present in the blend) was added after the heat treatment. In the latter case the MWP was dispersed along with lactose monohydrate in water at 5.5 g protein/100 g, 14 g total solids/100 g before addition to the pre-heated milk base. Each blend was replicated 3 times, for a grand total of 51 runs.

Even though the protein content was maintained constant, the necessary quantity of GDL varied, because of differences in calcium and phosphate contents between the studied milk protein ingredients, and hence different buffer capacities of the reconstituted milk protein blend. Preliminary mixture experiments (vertices, edges and center - a total of 7

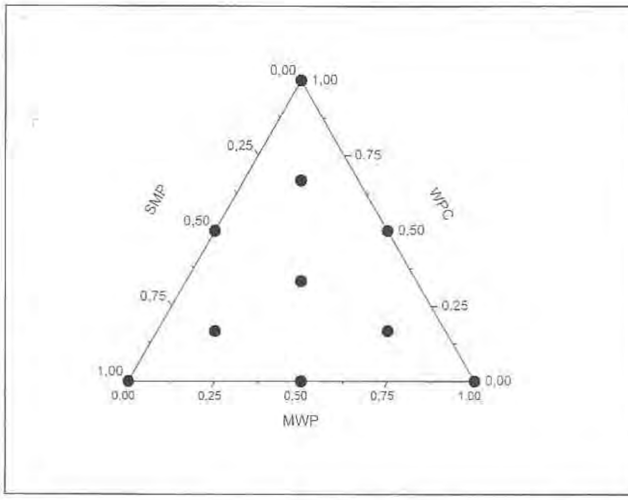

Fig. 1: Experimental design. Dots indicate fractions of SMP, MWP and WPC added, to a total of 2 per cent protein.

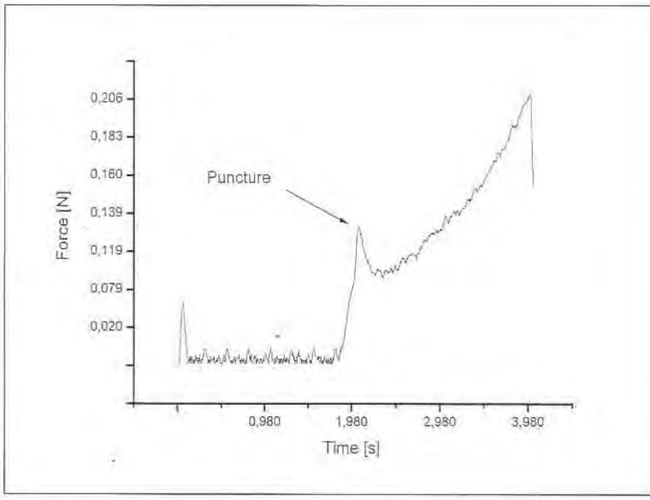

Fig. 2: Penetration test of milk gels. Puncture force is recorded at the first peak of the force-distance curve.

runs) were carried out to establish the amount of GDL to add as a function of the ratio of SMP, MWP and WPC. The following model resulted, with $R^2_{adjusted} = 0.9973$:

GDL to add (% w/w) = 1.92 SMP + 1.46 MWP + 1.51 WPC + 1.01 MWP WPC

The resultant gels were stored at 5°C for 24 h, and subsequently analyzed. A penetrometric method was employed to evaluate the firmess of the intact gels, using a TA-XT2i Texture Analyser (Stable Micro Systems, Godalming, UK). A cylindric ebonite probe of 10 mm diameter was used; the compression speed was 10.0 mm s^{-1}. Firmness was given as the puncture force (Fig. 2). The water holding capacity (WHC) of the gels was measured by centrifugation in a Sorvall RC6 centrifuge at 10,000 g and 10°C for 10 min. The supernatant was weighed ($m_{supernatant}$), and the water holding capacity calculated as:

$$WHC = \left(\frac{30 - m}{30}\right) \bullet 100\%$$

Firmness and WHC were modelled by PLS regression, including quadratic terms and two-way interactions.

Table 1: PLS model overview

Samples	Response	Latent Variables (LV's)	Explained variance (%)			
			LV 1	LV 2	LV 3	Total
MWP added						
before heat treatment	Firmness	3	51	31	9	91
	WHC	3	48	33	5	86
after heat treatment	Firmness	3	46	15	9	70
	WHC	3	46	34	2	82

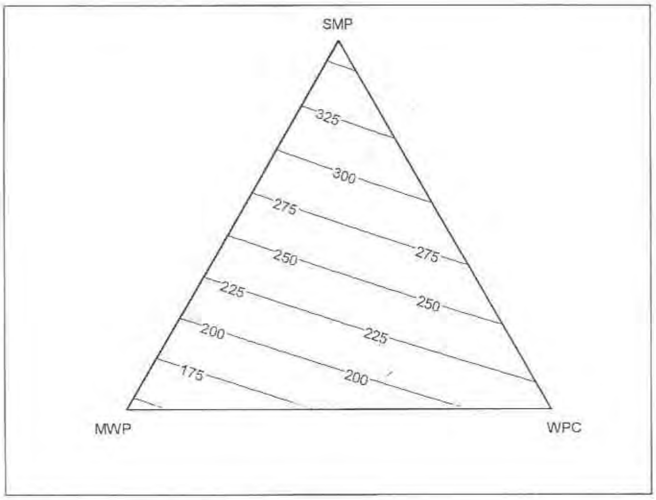

Fig. 3: Contour plot of predicted Firmness responses, with MWP added before heat treatment.

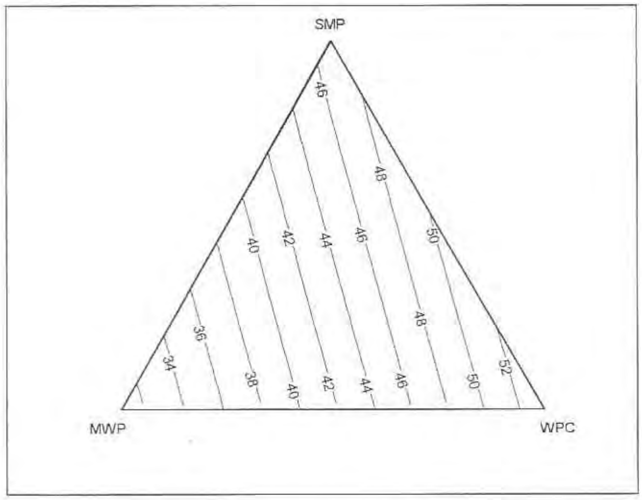

Fig. 5: Contour plot of predicted WHC responses, with MWP added before heat treatment.

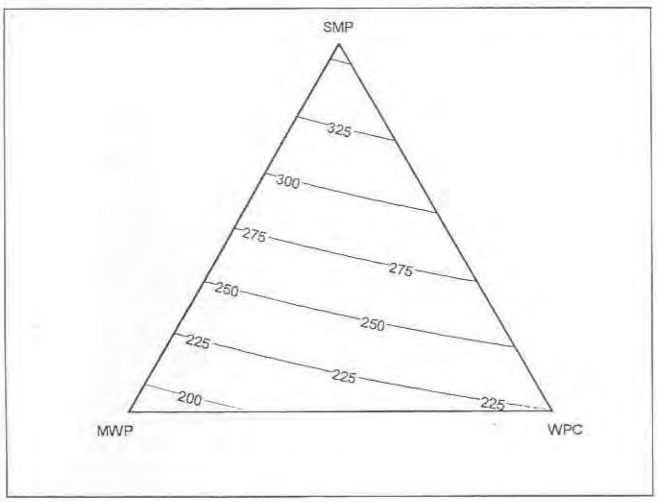

Fig. 4: Contour plot of predicted Firmness responses, with MWP added after heat treatment.

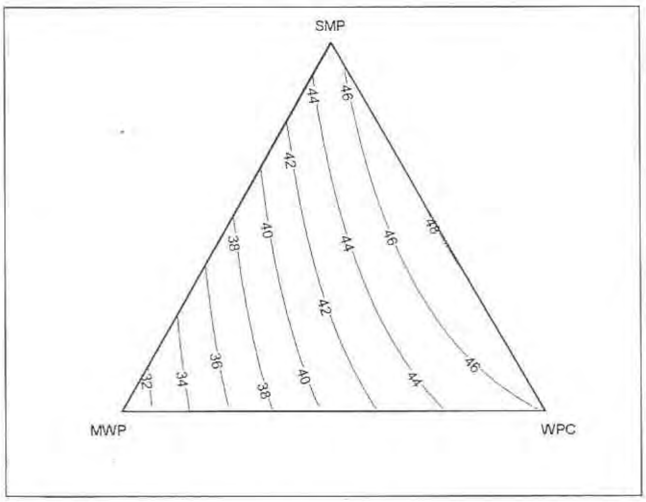

Fig. 6: Contour plot of predicted WHC responses, with MWP added after heat treatment.

Leave-one-out cross-validation, with replicate as cross-validation segment, was used to determine the number of latent variables.

3. Results

Both firmness and WHC could be modelled adequately by 3 latent variables, see Table 1

Regression coefficients of mixture models can be difficult to interpret (13). The resulting mixture models are thus represented graphically in Figs. 3-6 as contour plots of predicted responses. From Figs. 3 and 4 it is apparent that firmness is only slightly increased in the gels made from milk protein blends where MWP is added after pre-heat treatment. Water holding capacity, by contrast, is barely affected (Figs. 5 and 6).

The firmness was invariably highest in samples made with SMP, followed by MWP and WPC. WHC, by contrast, was highest for WPC, followed by SMP and MWP. These findings differ from those of (6), who found that WPC resulted in the highest syneresis; the methods of analysis employed are rather different, however.

There is only a slight curvature in the contour plots, which suggests a lack of interaction between the studied ingredients with regards to both firmness and WHC. Firmness and WHC were found to be poorly correlated ($r = 0.36$).

4. Conclusions

Even though the MWP is eminently capable of emulating the sensory perception of creaminess imparted by fat, it is clear that its effect on the microstructure of acid milk gels made from a pre-heated milk base is quite different from that of homogenized fat globules. Heat treatment had little effect on the functionality of microparticulated whey protein particles implying that they do not actively promote structure formation in acid milk gels, as do homogenized fat globules. It is conceivable that the sensory effect of MWP is more subtle, in that it affects the oral breakdown path in a similar way to homogenized fat globules or it may simply be matter of having approximately the same particle size

In either case, it might be possible to render the MWPs more reactive, and hence more directly integrated in the acid milk gel network by modifying the surfaces of these, i.e. by controlling the degree of denaturation, by coating with undenatured protein, or otherwise.

Acknowledgement

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Comparison of foaming capacity of caseinmacropeptide from different species with whey protein concentrate

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Foaming properties were analysed for whey protein concentrate (WPC) and compared with those of caseinmacropeptides (CMPs) isolated from cow, ewe and goat cheese whey. All CMPs showed a higher foaming capacity (140-180 mL) than WPC (120-150 mL) independent of conditions. These results could be affected by the presence of inhibitory compounds (fat and lactose) in the WPC, partially or totally removed during isolation procedure in the CMP and to the small molecular weight of the CMP peptide compared with the WPC. Foaming capacity of WPC and CMPs showed a significant dependence on pH and, to a lesser degree, on protein concentration and ionic strength. All the samples exhibited the highest foaming values at alkaline pH and minimum values at acid pH, increasing with protein concentration. Foam stability values were higher for CMP solutions than for WPC. The latter showed similar stability values as CMPs in acid pH conditions (<85%), however, at alkaline pH WPC lost more than 30% while CMPs showed no significant dependence on pH. Foam stability of the different CMP solutions could not be correlated with the variables pH, ionic strength and protein concentration; whereas pH and protein concentration influenced the stability of WPC foams.

Vergleich der Schäumungskapazität von Casein-Makropeptid unterschiedlicher Tierarten mit Molkenproteinkonzentrat

Die Schäumungseigenschaften von Molkenproteinkonzentrat (WPC) wurden untersucht und mit denen von aus Kuh-, Schaf- und Ziegenkäsemolke isolierten Caseinmakropeptiden (CMPs) verglichen. Alle CMPs zeigten unabhängig von den Bedingungen eine höhere Schäumungskapazität (140-180 mL) als WPC (120-150 mL) . Diese Ergebnisse könnten durch hemmende Stoffe (Fett und Laktose) im WPC, die teilweise oder ganz während des Isolierungsverfahrens von CMP entfernt werden, und durch das geringe Molekulargewicht des CMP-Peptids im Vergleich zum WPC verursacht werden. Die Schäumungskapazität von WPC und CMPs zeigte eine signifikante Abhängigkeit vom pH-Wert und in geringerem Maße von der Proteinkonzentration und der Ionenstärke. Alle Proben wiesen die höchsten Schaumwerte bei

Sensory, rheological and spectroscopic characterization of low-fat and non-fat cream cheese

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The present study explored relationships between physical/chemical and sensory properties in a set of 20 low-fat and non-fat cream cheeses. Experimental design variables were fat content (0, 3, 6 and 9%), pH (4.40 and 5.00) and salt content (0.4 and 0.9%). Additionally, two samples at 0% and 9% fat (centred pH 4.7 and salt 0.65%) were included in true replicates. Twenty-nine sensory descriptors were recorded, including the meta-descriptor *Creaminess*. High correlations were found between several descriptors, indicating that the systematic sensory variance in data was all closely related to the three underlying design variables. The sensory descriptor *Hand resistance* (i.e., tactile firmness during spreading of cheese on a model bread surface) was best predicted by squeezing flow viscometry (RMSEP=1.11 cm; a 15 cm scale was used) and shear viscometry (RMSEP=1.03 cm, excluding non-fat samples). Elongational viscosity as determined by contraction flow viscometry was on a par with dynamic oscillation in terms of predicting *Hand resistance* by linear regression (RMSEP=2.31 cm and 2.17 cm, respectively), but inferior to squeezing flow viscometry (RMSEP=1.80 cm). However, taking into account the measurement uncertainty, similar maximal correlations were found for contraction flow and squeezing flow (0.97 and 0.98, respectively). Spectroscopy proved useful in prediction of rheological properties.

Keywords: Cream cheese, sensory, squeezing flow, contraction flow, spectroscopy

1 INTRODUCTION

Compared to other dairy products, there are only few publications on cream cheese available in the public domain, with most of the information being proprietary to manufacturers. As is the case for other product groups, many low-fat and even non-fat cream cheeses have entered the market over the past years. In particular non-fat cream cheeses are difficult to characterize rheologically due to their tendency to fracture rather than flow above the yield stress [1]. Another problem is the presence of particles, which may render impossible the use of conventional rheological techniques such as steady shear viscometry. Among alternative methods described in the literature are a capillary extrusion technique [2-3] and a method employing a Brookfield viscometer with a spiral adapter [4]. Squeezing flow [5] and contraction flow [6] viscometry are two interesting candidates of a more fundamental nature which both, ideally, measure elongational properties; it has been suggested [7], but not yet verified experimentally, that elongational viscosity is more sensorially relevant that shear viscosty. A higher strain rate (but still much lower than that encountered in oral processing) is attained in contraction flow compared to squeezing flow viscometry. Squeezing flow viscometry has been evaluated for cream cheese [8]. The so-called imperfect squeezing flow setup was used, with an acrylic dish lubricated with silicone oil; from the biaxial flow curves the investigated cream cheeses could be adequately described as Power Law materials, with low-fat cream cheeses generally having higher values of n, and lower values of K. Few studies have dealt with sensory-structure relationships in cream cheese products. In one study, the microstructure was related qualitatively to manufacturing conditions and sensory properties [9]. We have studied the correlation between sensory and instrumental data taking into account the measurement error of both, in order to quantitate the true correlations. Finally, with a wiev to exploring the usefulness of Process Analytical Technologies in food texture studies, we have studied the use of spectroscopic techniques in predicting rheological properties, i.e. elastic moduli of cream cheese.

2 MATERIALS AND METHODS

2.1 Cream cheese manufacture

Cream cheeses were produced in a pilot plant according to a standard methodology. A milk base was homogenized, pasteurized, fermented, pH adjusted, concentrated by centrifugation, pasteurized, homogenized and hot-filled. A total of 20 samples were produced according to a 4 x 2 x 2 full factorial design with duplicated centre samples. The factors studied were pH (4.4 and 5.0), salt (0.4 and 0.9 g/100 g) and fat (0, 3, 6 and 9 g/100g). The center samples had pH 4.7 and 0.65 g salt/100 g, and either 0 or 9 g fat/100 g.

2.2 Sensory analysis

Sensory testing comprised a descriptive analysis by a trained panel (10 participants). Twenty-eight sensory descriptors were developed by consensus during eight hours of training sessions, using refererence samples where feasible. Tab. 1 lists the descriptors, their definitions, abbreviations and original terms in Danish. In addition, the descriptor *Creaminess* was evaluated without prior consensus among panellists, i.e. each panellist used his or her own concept of *Creaminess*.

Table 1: Sensory descriptors

Descriptor group	Descriptors
Aroma	*Cream, Acidic, Butter, Goat, Old milk*
Tactile	*Hand resistance*
Visual	*Whiteness, Greyness, Yellowness, Blueness, Glossiness, Grain concentration, Grain size*
Flavour and taste	*Goat flavour, Butter flavour, Sweet taste, Salt taste*
Textural	*Smoothness, Firmness, Flouriness, Chalkiness, Stickiness, Meltdown rate, Astringent, After-mouthfeel*
Meta-descriptor	*Creaminess*

**Grain concentration* and *After-mouthfeel* did not significantly discriminate between samples and were excluded from further analysis.

The samples were kept at 13ºC for one hour prior to the sensory sessions, and served in random order, one sample at a time, and ten samples of 40-45 g per session, under normal light conditions in transparent 100 ml containers with lids. Sensory analysis took place in a sensory laboratory complying with international standards for test rooms. Samples were scored on a computer screen using a 15 cm unstructured scale; a computerized score collection software (FIZZ, BIOSYSTEMES, Couternon, France) was employed. The scales were anchored with “a little” and “a lot”, except for the tactile descriptor Hand resistance, for which the terms “low” and “high” were used; Grain size was anchored with “small” and “large” and Meltdown rate with "slow" and "fast".

2.3 Rheological measurements

Steady shear viscometry was performed at 13ºC using a 15 mm parallel plate measuring system on a Bohlin C-VOR rheometer (Malvern Instruments Ltd., Malvern, Worcestershire, UK). A flow curve was recorded at 35 logarithmically spaced shear rates from 1.029 to 299 s^{-1}. Squeezing flow viscometry between parallel circular Teflon plates (d=100 mm) was performed using an Instron 5564 UTM (Instron Corp., High Wycombe, UK) with a 500 N load cell. Cold cream cheese (5ºC, 20 g) was spread on one plate, and compressed at a rate of 0.1 mm s^{-1} to a final height of 0.9 mm; the result of a squeezing flow measurement is a vector of extensional viscosities representing heights from 2.0 down to 0.9 mm. Contraction flow viscometry was performed at 13ºC using nozzles with a Hencky strain of 3.07, yielding a single value for extensional viscosity. The displacement rate was 1 mm s^{-1} (extensional strain rate 1.376 s^{-1}). Finally, dynamic oscillation was performed at 13ºC using a 15 mm parallel plate measuring system on a Bohlin C-VOR rheometer (Malvern Instruments Ltd., Malvern, Worcestershire, UK). Dynamic moduli were measured at 20 frequencies between 0.01-10 Hz, at a strain of 0.002. All rheological measurements were performed in triplicate.

2.4 Spectroscopic measurements

Fluorescence spectra (excitation-emission matrices) were measured at ambient temperature using a Perkin Elmer LS50 B spectrofluorometer equipped with a front-face accessory. Excitation and emission was varied from 260 and 260 nm to 360 and 600 nm, respectively. The VIS/NIR reflectance spectra were recorded on a NIR-Systems 6500 spectrophotometer (FOSS NIRSystems, Silver Spring, MD). Spectra were obtained by averaging 32 scans of the range 400-2500 nm; spectra were pre-processed using Multiple Scatter Correction as well as Extended Multiple Scatter Correction [10]. A circular sample holder (diameter 4 cm) was used for both fluorescence and VIS/NIR measurements.

2.5 Data analysis

Data analysis of sensory data was performed using uni-variate analyses (ANOVA for each descriptor). ANOVAs for the individual descriptors were performed using panelists as random factor. Multivariate data analysis (ANOVA Partial Least Squares Regression, [11]) was applied to investigate relationships between sensory data and the experimental design. After initial analysis, data were averaged over panellists, and those data were used for further analysis. For all the multivariate analyses, cross validation was performed, leaving each replicate out at a time Two data analytical approaches were followed to relate sensory and rheological data. One was the application of uni- and multivariate regression methods (PLSR), in which raw data (averages of triplicates) was regressed on averaged sensory descriptors, an approach which, in the case of PLSR, has been termed Spectral Stress-Strain Analysis in the literature [12]. Predictive performance was expressed in terms of Root Means Square of Cross-Validation (RMSECV). The other data analytical approach was to combine mixel model ANOVA with measurement error methodology in order to assess the uncertainty of the studied correlations [13]. Products were treated as fixed factors, and panellists, replicates as well as all second-order interactions were considered random. Excitation-emission matrices were decomposed by PARAFAC [14] and subsequently regressed on rheological parameters using multiple linear regression. All PLSR models were validated by leave-one-out cross validation.

3 RESULTS AND DISCUSSION

From the the correlation loading plot of sensory data (Fig. 1) it is apparent that several key sensory attributes were strongly correlated.

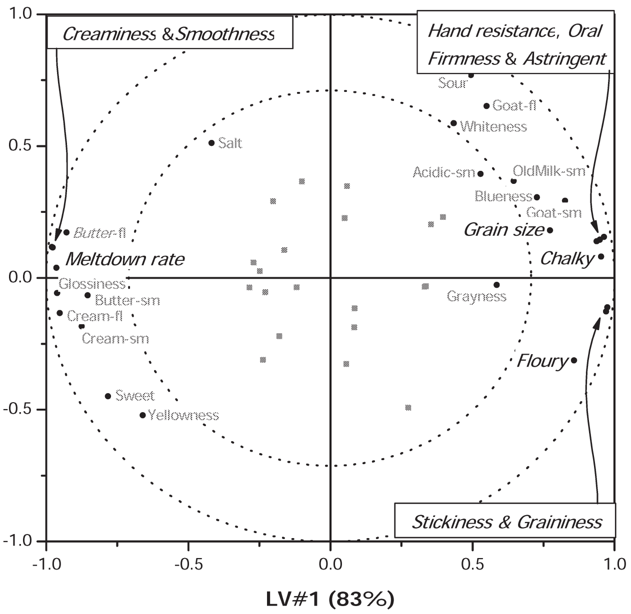

Figure 1: ANOVA-PLSR correlation loadings for the two first latent variables (LV). Sensory descriptors ● and Samples ■. Relevant textural sensory descriptors in *italics* The inner and outer circles represent 50% and 100% explained variance, respectively.

On particular note is the correlation between Creaminess and key textural attributes such as *Hand resistance* (r=-0.94), *Grain size* (r=-0.78), *Oral firmness* (r=-0.91), *Meltdown* (r=0.94), *Smoothness* (r=0.98), *Floury* (r=-0.90) and *Chalky* (r=-0.94). Detailed analysis showed that *Hand resistance* was the sensory descriptor thast best separated the samples, *i.e.* the combination of spanning most of the sensory scale (15 cm) and having the lowest uncertainty in panelists' evaluation. It was chosen as the one key sensory descriptor for further analysis of sensory-instrumental relationships. After averaging over panelists, the measurement uncertainty for *Hand resistance* was 0.96 cm (in terms of RMSECV). The size of the measurement uncertainty serves as a reference value for comparison of predictive ability of other data. The prediction can not possibly be better than the measurement error of the sensory descriptor.

Shear viscometry proved untenable for non-fat cream cheese because of its tendency to fracture rather than flow above the yield stress. For this reason, correction for shear flow in contraction flow viscometry was not possible for these samples, as this correction requires Power Law parameters as input. However, for the remaining low-fat samples it turned out that the difference was rather small (on the order of 5 per cent); it was thus decided not to correct for shear flow. Moreover, a certain degree of slip was apparent in the shear flow curves; in squeezing flow viscometry slip is not a problem, but rather a requisite.

The predictive ability of rheological raw data is given in Tab. 2-3.

Table 2: PLS modelling of *Hand resistance*.

Independent variable	Samples	LV	R^2	RMSECV
Squeezing flow	All	2	94.5	1.11
G'	All	1	73.5	2.18
Shear viscometry	Low-fat	2	85.9	1.03
Squeezing flow	Low-fat	1	84.3	0.95
G'	Low-fat	4	90.4	1.55

The multivariate PLS model indicate directly the which of the independent (X) variables carry the most weight in describing the dependent (Y; here: *Hand resistance*).

Table 3: Linear regression of rheological parameters on *Hand resistance*.

Independent variable	Samples	R^2	RMSECV
Squeezing flow	All	81.2	1.80
G' (10 Hz)	All	74.1	2.17
Contraction flow	All	66.3	2.31
Shear viscometry	Low-fat	69.1	1.34
Squeezing flow	Low-fat	84.2	0.99
G' (10 Hz)	Low-fat	51.5	1.63
Contraction flow	Low-fat	75.5	1.24

For example, the regression coefficients for the 1st latent variable of the model of squeezing flow data on *Hand resistance* point at the viscosity measure at the lowest height (0.9 mm) as the most informative variable (Fig. 2).

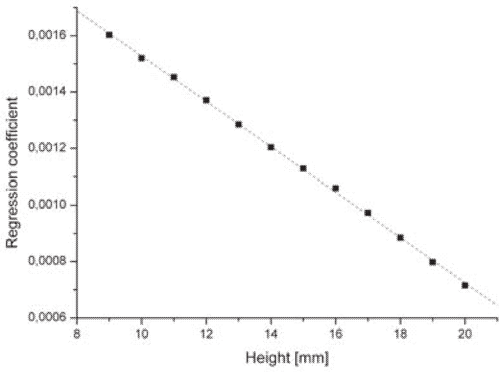

Figure 2: Regression coefficients, 1st latent variable, of PLS model of squeezing flow data on *Hand resistance*.

Comparing the PLS models (Tab. 2), where all the raw data was used, to the simpler, univariate regression models (Tab. 3) we notice that the PLS models fare better (in terms of RMSECV) than the univariate model in the case of squeezing flow viscometry. For other rheological data, e.g. elastic modulus this was not the case. This is because the elastic modulus raw data, i.e. the mechanical spectra, are more multicollinear than the squeezing flow raw data.

Comparing the predictive abilities of the different rheological data, we find, for both the entire dataset as well as for the subset excluding the non-fat samples that squeezing flow performs better than shear viscometry (in the case of the low-fat samples), which in turns performs better than elastic modulus, and lastly contraction flow. However, taking into account the measurement error of both the rheological and sensory data, we find that the true correlations are very similar for squeezing flow and contraction flow, both in terms of maximal and corrected correlations (Tab. 7). One should therefore be cautious in picking one method over the other.

From Tab. 4-6 we see that different spectroscopies provided good predictions of rheological parameters.

Table 4: PLS modelling of NIR spectra on elastic moduli (G').

Dependent variable	Samples	Pre-proces-sing	LV	r
G'	All	MSC	3	0.74
log[G']	All	MSC	4	0.84
G'	Low-fat	MSC	Poorly modelled	
log[G']	Low-fat	MSC	Poorly modelled	
G'	All	EMSC	2	0.74
log[G']	All	EMSC	2	0.78
G'	Low-fat	EMSC	3	0.69
log[G']	Low-fat	EMSC	3	0.65

Table 5: PARAFAC regression modelling of fluorescence spectra on elastic moduli (G').

Dependent variable	Samples	r
G'	All	0.36
log[G']	All	0.65
G'	Low-fat	0.83
log[G']	Low-fat	0.75
G'	All	0.44
log[G']	All	0.48
G'	Low-fat	0.35
log[G']	Low-fat	0.32

Table 6: PLS modelling of T_2 curves (low-field NMR) on elastic moduli (G').

Dependent variable	Samples	LV	r
G'	All	5	0.92
log[G']	All	6	0.93
G'	Low-fat	Poorly modelled	
log[G']	Low-fat	Poorly modelled	

Table 7: Maximal and corrected correlations between selected rheological (SF = squeezing flow, CF = contraction flow) and sensory parameters.

Sensory descriptor	Maximal correlations		Corrected correlations	
	SF	CF	SF	CF
Hand resistance	0.98	0.97	0.92	0.84
Oral firmness	0.98	0.97	0.88	0.86
Creaminess	0.99	0.98	-0.80	-0.80

4 CONCLUSIONS

Squeezing flow viscometry proved superior to shear viscometry in predicting the tactile firmness of low-fat and non-fat cream cheeses. Contrary to shear viscometry it could be used also on non-fat samples, and it predicted *Hand resistance* better. However, this could be specific to low-fat and non-fat cream cheese, and one should be cautious to ascribe this result to the fact that squeezing flow viscometry measures elongational rather than shear properties.

Measurement Error Methodology was useful in calculating the true correlation between sensory and instrumental variables. Its use is warranted when decisions on the usefulness of different rheological methods are to be made, but it goes without saying that selection bias should be avoided (i.e. preselecting the best rheological data).

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Effect of fat, protein and shear on graininess, viscosity and syneresis in low-fat stirred yoghurt

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The effect of fat (0.2, 0.85 and 1.5%) and protein (3.4, 4.4 and 5.4%) levels, as well as that of mechanical shearing (pore size of smoothing filter: 50, 100 and 150 µm) on the post-aggregation of stirred yoghurt particles as well as on the viscosity and syneresis in low-fat stirred yoghurt were investigated. To quantitate the proportion of yoghurt particles aggregated subsequent to the filtration of the stirred yoghurt gel we have introduced the Degree of Aggregation, i.e. the ratio of yoghurt particles with a particle size exceeding the filter size. Empirical models of Degree of Aggregation, as well as of viscosity and syneresis, were built using Response Surface Methodology (RSM). All three could be adequately modelled by including the fat and protein levels, their interaction term Fat x Protein as well as the filter pore size. Protein content and Degree of Aggregation were found to be positively correlated for fat levels above 0.7%, and negatively correlated for fat levels below 0.6%. The post-aggregation behaviour of low-fat, stirred yoghurt is ascribed to their branched network microstructure, which is presumed to be more susceptible to mechanical attrition.

Einfluss von Fett, Protein und Stress auf die Körnigkeit, Viskosität und Synärese von fettarmem gerührten Joghurt

Der Einfluss von Fett- (0,2, 0,85 und 1,5%) und Proteingehalt (3,3, 4,4 und 5,4%) sowie mechanischer Scherkraft (Porengröße des glättenden Filters 50, 100 und 150 µm) auf die Postaggregierung von gerührten Joghurtpartikeln, sowie auf die Viskosität und Synärese von fettarmem Joghurt wurde untersucht. Um den Anteil von aggregierten Joghurtpartikeln nach der Filtration zu beurteilen, wird ein Aggregierungsgrad vorgeschlagen, d.h. der Anteil von Joghurtpartikeln, deren Partikeldurchmesser die Porengröße übersteigt. Empirische Modelle von Aggregierungsgrad, Viskosität und Synärese wurden mit der Response Surface Methode (RSM) erstellt. Alle drei wurden zufriedenstellend modelliert durch Einschluss von Fett- und Proteinkonzentrationen, der Wechselwirkung Fett x Protein sowie der Filterporengröße. Proteingehalt und Aggregierungsgrad waren positiv korreliert bei Fettgehalten über 0,7% und negativ korreliert bei weniger als 0.6%. Das Postaggregierungsverhalten von fettarmem Joghurt wurde auf eine verzweigte Mikrostruktur zurückgeführt, die vergleichsweise empfindlich gegenüber mechanischen Einflüssen ist.

61 Yoghurt (composition and physical properties)

61 Joghurt (Zusammensetzung und physikalische Eigenschaften

1. Introduction

The presence of graininess (lumpiness, grittiness) is a common defect in dairy products found especially in low-fat, stirred semi-solid products such as yoghurt and cream cheese. These are essentially stirred acid milk gels, i.e. concentrated suspensions of acid gel particles in milk serum. The control of texture defects in cultured products has been reviewed recently (1). Graininess is commonly ascribed to the addition of whey protein. In one study, whey protein concentate (WPC) was partially substituted for skimmed milk powder (SMP) in lactose-reduced yoghurt (2). The resulting yoghurts were significantly grainier after one week of storage, implying that post-aggregation, i.e. the aggregation of acid milk gel particles occurring after the stirring of the gel, of the yoghurt particles play a part. Addition of WPC has been shown to result in more grainy yoghurts than SMP (2,3), even if the total solids (TS) is lower (2). Addition of caseinate gives an intermediate graininess and increasing the pre-heat treatment time of the milk base increases graininess in yoghurt (3). It was inferred that graininess is linked to the amount of denatured whey protein in the milk base. Additions of polysaccharides can also give rise to graininess due to phase separation (3), ostensibly by depletion flocculation. Addition of pectins, for instance, may cause graininess in stirred yoghurt if the added quantity exceeds the recommended 0.08-0.20 g/100 g. A different phenomenon is seen with modified starch; native starches tend to be shear-sensitive and are thus prone to cause graininess in stirred yoghurt. Graininess has also been found to depend on the starter culture (4); *L. delbrueckii var. bulgaricus* reportedly causes more graininess than *L. helveticus.* The notion of starter bacteria as the reason for graininess has, however, been dismissed by others (5). Increasing the intensity of stirring after acidification (changing the stirring speed from 50 to 400 ppm) gave a clear (negative) effect on graininess, whereas the stirring time did not. The fermentation time (achieved by using different starter cultures) has also been found to influence graininess, with r=0.86 (6,7). However, when modified stach was used, the situation appears to be different. In one study, yoghurt with added modified waxy maize starch, and fermented at 35ºC, was less grainy that when fermented at 43ºC, despite the longer fermentation time (8), whereas in another study it was found that an increasing substitution of SMP by WPC in modified starch-added yoghurt *reduced* the level of graininess (9); it should be noted that control samples without added starch did not exhibit graininess. In another study, the visual appearance of yoghurt changed from *very smooth* to *very granular* when the fermentation temperature was increased from 41 to 47ºC (10); it should be emphasized that some technological parameters (e.g. fermentation temperature and time) are confounded, and as such difficult to separate.

Grains recovered from yoghurt have been found to contain more protein and total solids, and little or no fat (5,7). Electron microscopy has not revealed any agglomerations of starter bacteria within the grains, lending credence to the notion that the starter cultures *per se* do not take part in the formation of granules. Graininess was found to be less pronounced when a more »viscous« (i.e., exopolysaccharide-forming) yoghurt starter culture was used.

With the specific objective of reducing graininess, the acidified milk gel is commonly submitted to a mechanical treatment (»smoothing«), in addition to the stirring process. This may consist of pumping the coagulum through a filter (11), and/or maintaining a constant backpressure over a valve (12).

Graininess has primarily been quantitated sensorially in the literature, *e.g*. by visual examination of a sample of yoghurt spread on dark glass (10). However, a method based on image analysis has also been decribed (13): a yoghurt sample is suspended in water and poured into a Petri dish; the number of grains with a diameter exceeding 1 mm is enumerated. One could argue that the limit of 1 mm should be set differently, since grains of a much smaller diameter can be perceived both visually and orally (14). In addition, soft, large grains might not contribute to the sensorially perceived graininess.

It is clear from the above that the level of graininess is influenced by the level of fat and protein, which also affect other functional characteristics such as viscosity and syneresis. A balance between the latter and graininess will thus have to be struck. However, the effects on graininess of fat and protein levels as well as the mechanical treatment of yoghurt (and, in particular, the interactions between these) have not been studied systematically. The purpose of the present study was to provide empirical response surface models of graininess as well as viscosity and syneresis in low-fat stirred yoghurt. Mathematical optimization (i.e., minimization) of graininess with viscosity and syneresis constraints will be dealt with in a forthcoming paper.

2 Materials and methods.

2.1 Experimental design

A three-level factorial design with four centre points was used. The factors considered were fat content (0.2, 0.85 and 1.5 per cent), protein content (3.4, 4.4 and 5.4 per cent) and filter pore size (50, 100 and 150 µm). A total of 30 samples of yoghurt were prepared in randomized order in the course of two days, including all combinations of fat content, protein content and pore size, as well as four centre points (0.85% fat, 4.4% protein and filter with 100 µm in poresize). Two centre point samples were manufactured each day.

2.2 Yoghurt manufacture

Pasteurized skimmed milk was standardized to the desired fat at protein contents by addition of pasteurized full cream (Arla Foods, Viby, Denmark) and medium-heat skimmed milk powder (Arla Foods Ingredients, Viby, Denmark), respectively. The ingredients were mixed using a Silverson L4R mixer (Silverson Machines Ltd., Waterside, UK) at 5℃. The milk base was homogenized at 200/50 bar, 65℃, and subsequently heat treated at 95℃ for 5 min. The milk was cooled to 42℃ and inoculated with yoghurt starter (YC-183, Chr. Hansen, Hørsholm, Denmark). Upon reaching pH 4.55 the yoghurt was cooled to 22℃ in a plate heat exchanger and smoothed by passing a tubular filter with a defined pore size (50, 100 or 150 µm) at a constant counterpressure of 2 bar. The yoghurt was kept at 5℃ for one week before analysis was performed.

2.3 Instrumental analyses

The particle size distribution of the yoghurt was measured using a Malvern Mastersizer Microplus (Malvern Instrument Ltd., Malvern, UK). Deionized water was used as solvent (identical results were found when reconstituted permeate powder was used). Before sampling the yoghurt was gently stirred with a spoon five times. For further analysis the proportion of particles (volume percentage) exceeding the pore size in the filter used to smoothen the yoghurt was used (see Fig. 1). This quantity, calculated by interpolation, was referred to as *the Degree of Aggregation* (DOA).

Syneresis was quantified by submitting yoghurt (30-32g) to centrifugation at 222 **g** for 10 min. at 4℃ (15), The clear supernatant was poured off, weighed and recorded as syneresis (%).

The viscosity was measured using a Brookfield viscometer (Model DV–III+, Brookfield Engineering, Stoughton, MA). The measurements were performed using a DT-bar spindle at 5 rpm, 10ºC. The Brookfield viscosity so obtained has been found to correlate well to sensory thickness (16).

All instrumental measurements were performed in triplicate, and mean values were used for further analysis.

2.4 Statistical analysis

In the statistical analysis all the factors were considered quantitative, except for the factor *pore size* used in the analysis of DOA, which was considered qualitative. A second order polynomial including interactions was used as initial model and step-wise regression was used to eliminate insignificant terms ($p > 0.05$) successively, simplifying the model.

3. Results

The four centre samples were used to test repeatability and day-to-day variability statistically. Using the response variable syneresis, we found that both replicate and day were not significant (p=0.33 and 0.22, respectively).

3.1 Degree of Aggregation

The extent of post aggregation occurring in the container after shearing, expressed as Degree of Aggregation (DOA) significantly depended on both the fat (p=0.0004) and the protein contents (p=0.042), with R^2_{adj}=0.97. In Fig. 2 the response surface of DOA as a function of fat and protein contents is presented. These results indicate that fat in the yoghurt matrix interfered with the post aggregation process, as a higher fat content resulted in a decreased DOA. The protein content appeared to interact with the fat content (p=0.0023). The results indicate that protein content and DOA are positively correlated for fat levels above 0.7%, and negatively correlated for fat levels below 0.6%. Results are given in Table1.

3.2 Viscosity

Statistical analysis showed that the fat content, the protein content and the interactions between them had a significant effect on the viscosity of the yoghurt with R^2_{adj}=0.92 (Table 2). Log-transformation of viscosity was necessary to achieve variance homogeneity. Filter size was found to influence the viscosity of the yoghurt as well. Homogenization of the milk base incorporates the milk fat into the protein matrix, where the protein covered fat globules act as pseudocasein micelles (17,18). When describing the character of the stirred yoghurt with respect to syneresis and viscosity, the role of fat can generally be compared to that of protein; homogenization of the fat increases the number of structure building components, which results in higher viscosity and lower syneresis (17). For yoghurts with a high level of protein, the character of the fat in the yoghurt matrix appeared to change. The present results indicate that for protein levels above 5.2% the fat content was negatively correlated to the viscosity (see Fig. 3).

3.3 Syneresis

The results from investigation of syneresis in yoghurt could be modelled (R^2_{adj}=0.92) with the significant parameters given in Table 3. The response surface of the predicted values for syneresis is depicted in Fig. 4. No significant interactions between the factors fat content and filter size, nor between protein content and filtersize were found. The minimum syneresis was found at a fat content of 1.5%, a protein content of 5.4% and a filter size of 91 µm. The main factor influencing syneresis was the protein content, supporting previous research (1,19).

4. Conclusions

The present results corroborate those of other researchers with regards to the influence of fat and protein content on syneresis and viscosity of yoghurt (1,17,18). However, some novel conclusions are proposed. For high levels of protein, a negative correlation between fat content and viscosity was registered. Assuming that protein is able to form a gel matrix by itself, the homogenized fat will compete for protein, because of the need for protein as membrane material for the newly formed homogenized fat globules. It is assumed that the homogenized fat globule, as a pseudocasein micelle, contributes to the structure formation to a lesser extent than the same amount of protein would do in the milk plasma. In this case, the protein used as membrane material is less efficient in structure formation, even though fat is incorporated into the matrix. Microstructure studies of low fat set style yoghurts shows a more branched network structure for low-fat yoghurts, than for yoghurts with a higher fat content, where the structure is more dense, and branched to a lesser extent (20). When shearing yoghurt, a branched network will be more prone to shattering than a dense, clustered network. Hence, a yoghurt with a branched network will have a greater potential for post-shearing aggregation in the container due to more broken bonds, which are expected to have a higher post-aggregation potential. The fat globules in the sheared yoghurt matrix are presumed to play a role as structure breakers, where they, due to difference in size, interfere with the aggregation in the container. Fat globules in a sheared yoghurt are thus assumed to play a dual role, where they a) promote formation of a dense, clustered network during fermentation, and b) interfere in the post-shearing aggregation in the container. This theory could explain the negative correlation between fat content and DOA. DOA was found to be a useful tool in investigation of the post-shearing textural alterations occurring in fermented milk. Correlation between sensory graininess and DOA was not investigated in the present work. However is seems reasonable that grains and sandy texture in stirred fermented milk to some extent can be related to the particle size distribution and thereby to DOA.

Acknowledgements

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Table 1: Parameter estimates (Est±SEM) for the dependent variable degree of aggregation (DOA). *p*-values are for parallel tests in the final model.

Model term	Degree of aggregation	p-value
Fat	-27.1±6.5	0.004
Protein	-3.13±1.5	0.042
Fat*protein	4.96±1.5	0.002
Filter 50	59.5±6.6 a1	
Filter 100	23.9±6.6 b	<0.0001
Filter 150	22.2±6.6 b	

Any two data in the column having a common letter are not significantly different (tested by t-test)

Table 2: Parameter estimates (Est±SEM) for the dependent variables log(viscosity). *p*-values <0.05 are for parallel tests in the final model, *p*-values>0.05 are for successive tests in the statistical model reduction		
Model term	log(viscosity)	p-value
Intercept	2.06±0.42	
Fat	0.417±0.11	0.0013
Protein	0.798±0.19	0.0004
Filter	$-4.40\cdot10^{-3}\pm1.7\cdot10^{-3}$	0.019
Fat^2	-	0.98
$Protein^2$	$-5.68\cdot10^{-2}\pm2.2\cdot10^{-2}$	0.015
$Filter^2$	$1.92\cdot10^{-5}\pm8.6\cdot10^{-6}$	0.036
Fat*protein	$-8.10\cdot10^{-2}\pm2.5\cdot10^{-2}$	0.004
Fat*filter	-	0.21
Protein*filter	-	0.65

Table 3: Parameter estimates (Est±SEM) for the dependent variables syneresis. *p*-values<0.05 are for parallel tests in the final model, *p*-values>0.05 are for successive tests in the statistical model reduction.		
Model term	Syneresis	P-value
Intercept	65.8±4.9	
Fat	-20.0±4.1	<0.0001
Protein	-9.39±0.91	<0.0001
Filter	$-0.146\pm6.2\cdot10^{-2}$	0.027
Fat^2	-	0.46
$Protein^2$	-	0.81
$Filter^2$	$8.06\cdot10^{-4}\pm3.1\cdot10^{-4}$	0.015
Fat*protein	2.89±0.91	0.004
Fat*filter	-	0.28
Protein*filter	-	0.17

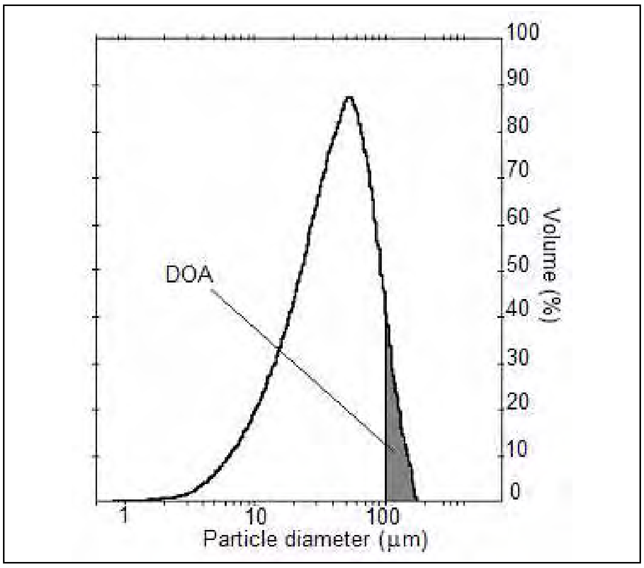

Fig. 1: Definition of Degree of Aggregation (DOA).

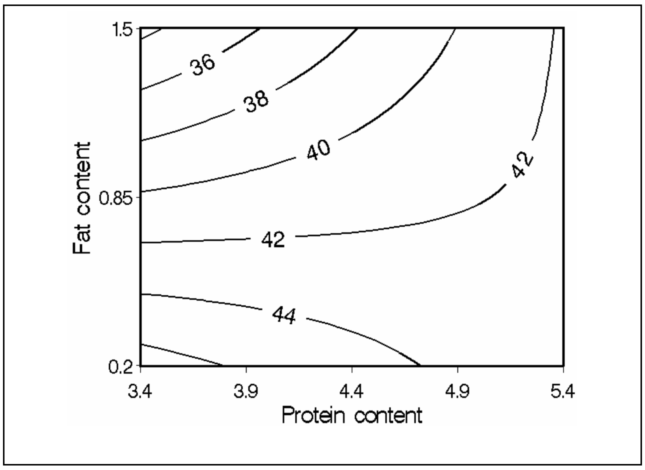

Fig. 2: Contour plot of the Degree of Aggregation as a funcion of fat and protein content.

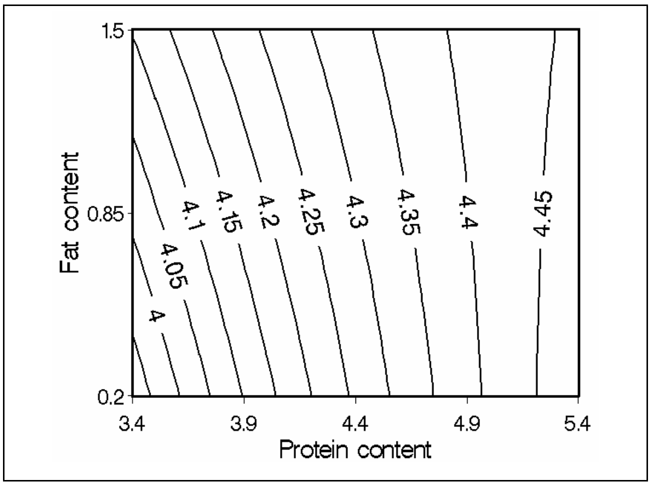

Fig. 3: Contour plot of log(viscosity) as a function of fat and protein content.

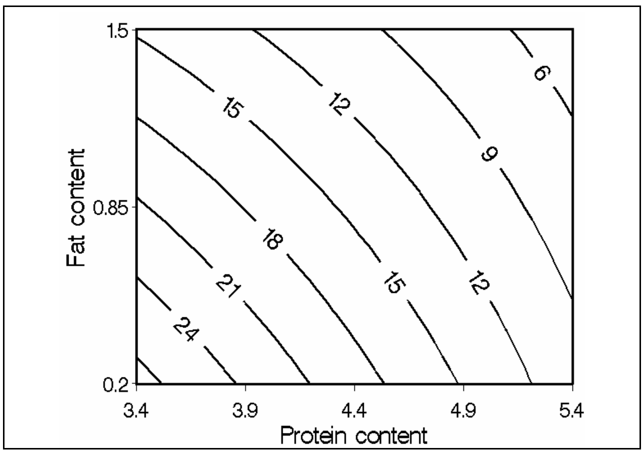

Fig. 4: Contour plot of syneresis as a function of fat and protein content

Prediction of sensory properties of low-fat yoghurt and cream cheese from surface images

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Abstract

The sensory properties of 25 plain yoghurts and 18 low-fat cream cheeses were investigated by descriptive analysis. In addition, digital images of sample surface were captured and the relationship between image properties and sensory properties were investigated. Global image features of the yoghurt and cream cheese surfaces were extracted using the Angle Measure Technique (AMT). Multivariate data analyses (Partial Least Squares Regression) were applied for investigation of the relation between digital image global features and sensory properties. For both product categories properties could be predicted with Root Mean Square Error of Cross Validation (RMSECV) for the yoghurts [1.00;1.97] and for the cream cheeses [0.29;2.00]. In both cases the largest RMSECV is for the prediction of *Creaminess*. Furthermore, other sensory descriptors, not related to appearance and structure could also be predicted. However, this is due to covariation with the visual descriptors.

Keywords

Yoghurt, Cream cheese, Sensory descriptive analysis, Creaminess, Angle Measure Technique, Image analysis, Multivariate data analysis, Partial Least Squares Regression

Introduction

Texture derives from the structure of food and the way ingredients interact as discussed by Wilkinson *et al*. (2000) and the perception of texture is a combination of information from several senses. The first step in sensory evaluation of food is most often the appearance, which is also the step with the shortest response time (Jones & O'Neil, 1985). Visual cues such as colour, gloss, grains and heterogeneity provide information about the surface properties of a food. The appearance provides the initial texture analysis of the product and sensory expectations of oral texture properties are created based on previous knowledge and encounters with other more or less similar products. Additional information is obtained by handling the food, e.g. stirring, cutting or spreading. However, the most important part of the dynamic texture perception occurs in the mouth. During mastication textural parameters are perceived when the food is broken into small particles by chewing, wetted and lubricated with saliva, and formed into a bolus suitable for swallowing. Hutchings and Lillford (1988) suggested that each food has a characteristic 'breakdown path' in the mouth comprised of three dimensions: breakdown of structure, degree of lubrication and time. Perception of a given food is a result of sensory input through all the senses, interpreted by the mind and influenced by personal experience. If the psychological element of expectation based on surface appearance or past experience is not met during the oral perception of the food, it can have a strong influence on reducing the level of texture acceptance (Szczesniak, 2002).

Surface characteristics – appearance – provide important textural cues (Ball *et al.*, 1957). Smoothness relates to the surface texture perception produced by moisture and/or fat content, whereas graininess relates to size, shape and arrangement of particles (ISO-11036, 1994; Szczesniak, 1963). Rohm *et al*. (1994) observed a close relationship between appearance of yoghurt surfaces and perceived mouth feel. A smooth mouthfeel correlates with a uniform surface whereas an irregular surface has a grainy texture. When assessing yoghurt surface properties, visual gloss - a measure of light reflectance - is an important attribute. According to Beck and Prazdny (1981) there are two types of light reflectance. The mirror-like shine perceived when an actual image of the light source appears on the product surface and diffuse reflectance where the light reflected is scattered by the product surface. They reported that highlights produce perception of glossiness, if an intensity gradient causes the surface to appear curved. However, one should be aware that luminance have been reported to be highly correlated with changes in the visible surface (Lederman & Abbott, 1981). Still, there is good evidence of high correlation between appearance and textural properties, thus making prediction of oral texture and mouth feel properties from surface images feasible. Little has been published on instrumental measurements of surface properties of acid milk gels such as yoghurt and cream cheese.

Creaminess is essential to many dairy products as it relates positively to product liking (Richardson-Harman *et al.*, 2000). Consumers seem to consider a product creamy when it has a high fat content, has dairy flavour and a viscous, slippery, greasy and mouth coating texture (Richardson-Harman *et al.*, 2000). Several researchers have found that creaminess relates both to thickness (depending on physical viscosity) and smoothness (depending on physical frictional forces) (Guinard & Mazzucchelli, 1996; Kokini & Cussler, 1983; Richardson *et al.*, 1993). Other studies have found that creaminess is highly correlated to perceived fattiness in different dairy product categories (Frøst *et al.*, 2001; Hyvönen *et al.*, 2003). Some studies have shown that creaminess is more complex, depending not only on texture characteristics, but also on flavour (de Wijk *et al.*, 2004). Research on the perception of fat in milk suggested a so-called meta-descriptor, 'total fattiness' to describe the overall sensory properties of fat in milk (Frøst *et al.*, 2001). The nature of a meta-descriptor is that it consists of a specific combination of a number of other more simple or straightforward descriptors[1]. Results from (Frøst *et al.*, 2001) suggest that the use of the meta-descriptor 'total fattiness' is appropriate, as this descriptor alone best preserve the data structure from the full set of descriptors (Dijksterhuis *et al.*, 2002), *i.e.* it is the descriptor carrying the highest amount of information, and best separates the different products under examination. We suggest that creaminess is a meta-descriptor as well.

In the present study, global image features were extracted using the Angle Measure Technique (AMT). This technique was originally introduced as an alternative method to fractal analysis (Andrle, 1994). It allows extraction and quantification of global properties of and image. The technique has subsequently been used for feature extraction in images of several types of food: bread texture (Esbensen *et al.*, 1996; Kvaal *et al.*, 1998); model dressings (Egelandsdal *et al.*, 1999); mayonnaise (Indahl & Næs, 1998); and some non-food applications: powder (Huang & Esbensen, 2000;2001). A thorough investigation of the methodology of the AMT principle and software is currently being performed (Johansen *et al*., 2006). Preliminary results indicate that AMT is most effective as analysis tool for isotropic images – which is the case with images of yoghurt and cream cheese surfaces. The present study investigates the relation between sensory properties (concentrating on structure-related properties - appearance, mouth feel and perceived texture) and surface

[1] Naming the phenomenon a meta-descriptor should be credited to Garmt Dijksterhuis.

structures of plain yoghurt and cream cheese. Of particular interest is the prediction of creaminess from surface images.

Materials and methods

Experimental design and product manufacture

Yoghurt

A total of 25 plain stirred yoghurts were produced and analysed in a factorial design as indicated in table 1. The total fat content was adjusted by addition of full fat cream (40% milk fat) to the milk base. The yoghurts were produced according to standard methodology for manufacture of stirred yoghurt (blending, pre-pasteurisation (65°C), homogenization (200bar), pasteurisation (95°C for 5 min), cooling (42°C), inoculation (YC-183, Chr. Hansen A/S, Denmark), incubation (below pH value 4.6), cooling (22°C), mixing, filling and final cooling (below 10°C)). The fermentation conditions were kept constant (final pH value 4.10-4.30). All yoghurts were stored at 4°C for exactly one week before further analysis, ensuring similar structural development. The yoghurts were produced and analysed in three replicates, except for the reference which was produced each day and analysed in 12 replicates. For each session, six different yoghurt and one reference sample was photographed and analysed by descriptive sensory analysis.

Table 1. The 25 analysed yoghurts abbreviations and composition. The different contents of fat (0, 1, 3) and protein (0, 1, 2, 3, 4) added and a short description of these proteins (N, S, C, V, M).

Product abbreviations	Fat content (%)			Added protein type	Total protein level (w/w%)				
	0	1	3	(N, S, C, V, M)	0	1	2	3	4
A0-N-0	0.3			None (N)	3.3				
B1-N-0		1.5			3.3				
C3-N-0			3.5		3.3				
D0-S-2	0.3						4.8		
E0-S-3	0.3			Skim milk powder (S)				5.4	
F1-S-2		1.5					4.8		
G1-S-3		1.5						5.4	
H0-C-1	0.3					4.2			
1-4 0-C-2*	0.3						4.8		
I0-C-3	0.3			Commercial whey protein				5.4	
J1-C-1		1.5		concentrate (C)		4.2			
K1-C-2		1.5					4.8		
L1-C-3		1.5						5.4	
M0-V-1	0.3					4.2			
N0-V-2	0.3						4.8		
O0-V-3	0.3			High viscosity producing whey protein				5.4	
P1-V-1		1.5		concentrate (V)		4.2			
Q1-V-2		1.5					4.8		
R1-V-3		1.5						5.4	

S0-M-2	0.3			4.8		
T0-M-3	0.3				5.4	
U0-M-4	0.3		Microparticulated whey protein concentrate (M)			6.0
V1-M-2		1.5		4.8		
X1-M-3		1.5			5.4	
Y1-M-4		1.5				6.0

* The yoghurt with 0.3% fat added commercial whey protein concentrate adjusted to 4.8% total protein was selected as the reference to appear in all 12 sensory sessions. Due to analytical conditions these 12 samples were treated as 4 different products.

Cream cheese

A total of 18 different cream cheeses and replicates of two were produced and analysed in a factorial design as indicated in table 2. The cream cheeses were produced in a pilot plant, according to standard methodology for manufacture of cream cheese. A milk base was homogenised, pasteurised, fermented pH adjusted, concentrated by centrifugation, pasteurised, homogenised, hot-filled and cooled.

Table 2. The 20 analysed cream cheeses abbreviations and composition. The different contents of fat (0, 3, 6, 9), salt content (1, m, 2) and pH value (1, m, 2).

Product abbreviations	Fat content (%)				Salt content (%)			pH value		
	0	3	6	9	1	m	2	1	m	2
A-F0-S1-p1	0.0				0.4			4.4		
B-F0-S1-p2	0.0				0.4					5.0
C-F0-S2-p1	0.0						0.9	4.4		
D-F0-S2-p2	0.0						0.9			5.0
E-F3-S1-p1		3.0			0.4			4.4		
F-F3-S1-p2		3.0			0.4					5.0
G-F3-S2-p1		3.0					0.9	4.4		
H-F3-S2-p2		3.0					0.9			5.0
I-F6-S1-p1			6.0		0.4			4.4		
J-F6-S1-p2			6.0		0.4					5.0
K-F6-S2-p1			6.0				0.9	4.4		
L-F6-S2-p2			6.0				0.9			5.0
M-F9-S1-p1				9.0	0.4			4.4		
N-F9-S1-p2				9.0	0.4					5.0
O-F9-S2-p1				9.0			0.9	4.4		
P-F9-S2-p2				9.0			0.9			5.0
Q-F0-Sm-pm	0.0					0.65			4.7	
R-F0-Sm-pm	0.0					0.65			4.7	
S-F9-Sm-pm				9.0		0.65			4.7	
T-F9-Sm-pm				9.0		0.65			4.7	

The cream cheeses (Q, R, S, T) with the lowest and highest fat content adjusted to both average salt content and pH-level were selected as the reference to appear twice.

Sensory descriptive analysis

Yoghurts

A panel consisting of 12 external paid panelists was used for the evaluation. All panelists had passed screening tests according to ISO-standards (ISO-8586-1, 1993), and had previous experience with sensory evaluation. Training (5 sessions of approximately 1½ hour) as well as the final assessment followed the recommendations of the International Dairy Federation (International IDF Standard 99C, 1997). A vocabulary best describing the yoghurts was developed on the basis of IDF standards (IDF, 1997), earlier used terms (Muir & Hunter, 1992; Szczesniak, 2002) and the panellists' own words. The final 29 descriptors and their definitions can be seen in table 3. Idiosyncratic definition of the meta-descriptor *Creaminess* was allowed, to facilitate the elucidation of differences among panellists in their evaluation. Sensory descriptive analysis (three true replicates, 12 sessions in total) were carried out in the sensory laboratory at the Royal Veterinary and Agricultural University, which comply with international standards for test rooms (ASTM, 1986; ISO-8589, 1988). All samples were served in plastic containers with lids, and coded with three-digit random numbers. Samples were served in a balanced randomised order. All samples contained approximately 100 ml of yoghurt, and were stored at 13°C in a controlled temperature cabinet for about 1 hour before serving (IDF, (1997))

All assessments were collected on a computerised data collection system (FIZZ v.2.10a Biosystemes, Couternon, France). The order of evaluation of the descriptors was as follows: aroma, appearance, flavour and taste, texture and finally manipulation by spoon. All descriptors were evaluated on a 15 cm unstructured line scale anchored at the left end with "a little", "slow" or "thin" (in Danish: "lidt", "langsom" or "tynd") and at the right end with "a lot", "fast" or "thick" (Danish: "meget", "hurtig" or "tyk"), depending on the character of the descriptor. All sensory evaluations were performed in individual booths, where tap water and unsalted crackers (flatbrød) were available.

Cream Cheese

A panel consisting of 10 external paid panelists was used for the evaluation. All panelists had passed screening tests according to ISO-standards (ISO-8586-1, 1993), and had previous experience with sensory evaluation. The training and assessments followed the same recommendations as the yoghurt study and a vocabulary consisting of 29 descriptors were used (table 3).

Both the training and the descriptive analysis were performed in the same manner as the yoghurt study, except that all samples contained 40-45 gram of cream cheese. The descriptive analysis consisted of 6 sessions with three replicates (10 samples evaluated in each session). The descriptors were evaluated in the following order: aroma, non-oral manipulation, appearance, flavour and taste, texture and mouth feel. The only change in the anchor points on the unstructured line scale were that to the left end “small” (in Danish: “lille”) and at the right end “large” (Danish: “stor”) were used.

Table 3. Sensory descriptors for yoghurt and cream cheese, their definitions and original words in Danish.

Descriptors	Definition (reference material)	Anchor points	Original Danish words	Product	
Aroma			*Lugt*	*Yoghurt*	*Cream cheese*
Tomato smell	Intensity of tomato aroma (0.3 L yoghurt (Jersey 0.1% fat, Thise Dairy, Denmark) added 5 drops of Heinz ® Tomato Ketchup)	a little – a lot	Tomatlugt	√	
Lamb smell	Intensity of lamb aroma (see below for detailed procedure*)	a little – a lot	Lammelugt	√	
Cream smell	Intensity of raw cream aroma (full fat homogenised milk (3.5% fat) and cream (38% fat) in a 1 to 5 mixture)	a little – a lot	Flødelugt	√	√
Buttermilk smell	Intensity of buttermilk aroma (Organically produced buttermilk (ArlaFoods, Denmark))	a little – a lot	Kærnemælkslugt	√	
Flour smell	Intensity of flour aroma (0.3 L yoghurt (Jersey 0.1% fat, Thise Dairy, Denmark) added 15 mL wheat flour)	a little – a lot	Melet lugt	√	
Acidic smell	Intensity of acidic smell when opening the sample	a little – a lot	Syrlig lugt		√
Butter smell	Intensity of butter flavour (Lump of organically produced old fashioned churned, salted butter (Lurpak ®, ArlaFoods, Denmark))	a little – a lot	Smørlugt		√
Goat smell	Intensity of goat–like aroma (goat yoghurt)	a little – a lot	Gedelugt		√
Old milk smell	Intensity of old milk aroma	a little – a lot	Gammel mælk lugt		√
Non-oral manipulation			*Manipulation med hånd*		
Resistance	Resistance during spread with a knife	low - high	Modstand		√
Appearance			*Udseende*		
Whiteness	Intensity of the colour white	a little – a lot	Hvid farve	√	√
Greyness	Intensity of the colour grey	a little – a lot	Grå farve	√	√
Greenness	Intensity of the colour green	a little – a lot	Grøn farve	√	
Yellowness	Intensity of the colour yellow	a little – a lot	Gul farve	√	√
Blueness	Intensity of the colour blue	a little – a lot	Blå farve		√
Glossiness	Degree of surface shininess	a little – a lot	Blankhed	√	√
Graininess	Degree of yoghurt surface graininess	a little – a lot	Grynethed	√	
Grain concentration	Evaluation of closeness of grains	a little – a lot	Koncentration af gryn		√
Grain size	Evaluation of the average size of grains	small – large	Størrelse af gryn		√
Flavour and			*Smag*		

taste					
Lamb flavour	Intensity of lamb flavour (see above)	a little – a lot	Smag af lam	√	
Goat flavour	Intensity of goat flavour (see above)	a little – a lot	Smag af ged		√
Butter flavour	Intensity of butter flavour (Lump of organically produced old fashioned churned, salted butter (Lurpak ®, ArlaFoods, Denmark))	a little – a lot	Smag af smør	√	√
Cream flavour	Intensity of cream flavour (see above)	a little – a lot	Smag af fløde	√	√
Buttermilk flavour	Intensity of buttermilk flavour (see above)	a little – a lot	Smag af kærnemælk	√	
Flour flavour	Intensity of flour flavour (see above)	a little – a lot	Melet smag	√	
Sour taste	Intensity of sour taste	a little – a lot	Sur smag	√	√
Sweet taste	Intensity of sweet taste	a little – a lot	Sød smag	√	√
Salt taste	Intensity of salt taste	a little – a lot	Salt smag		√
Texture and mouthfeel			*Konsistens*		
Viscosity	Perceived thickness of the sample evaluated in the mouth	thin – thick	Viskositet	√	
Smoothness	Perceived smoothness of the sample evaluated in the mouth	a little – a lot	Glathed	√	√
Firmness	Perceived firmness of the sample evaluated in the mouth	a little – a lot	Fasthed		√
Flouriness	Intensity of flour aroma (0.3 L yoghurt (Jersey 0.1% fat, Thise Dairy, Denmark) added 15 mL wheat flour)	a little – a lot	Melethed		√
Chalkiness	Perceived chalkiness of the sample evaluated in the mouth	a little – a lot	Kridtethed		√
Graininess	Perceived graininess of the sample evaluated in the mouth	a little – a lot	Grynethed		√

Table 4 continued. Sensory descriptors for yoghurt and cream cheese, their definitions and original words in Danish.

Stickiness	Perceived stickiness of the sample evaluated in the mouth	a little – a lot	Klistrethed		√
Meltdown rate	Amount of "work" to break down the bolus	slow – fast	Nedsmeltning	√	√
Astringent	Intensity of saliva losing feeling in the mouth – using the tough against the palate or the back of the teeth	a little – a lot	Astringerende	√	√
Fatty after mouthfeel	Degree of "fatty" mouth coating after expectoration of the sample	a little – a lot	Fedtet eftermundfylde	√	
Dry after mouthfeel	Degree of mouth dryness after expectoration of the sample	a little – a lot	Tør eftermundfylde	√	
Aftermouthfeel	Degree of mouth coating after expectoration of the sample	a little – a lot	Eftermundfylde		√
Non-oral manipulation			*Manipulation med ske*		
Non-oral viscosity	Rate of a spoon full to blur when it is placed on top of the sample	a little – a lot	Gelstivhed	√	
Graininess on lid	Half a spoon of sample spread on a lid	a little – a lot	Grynethed på låg	√	
Viscosity with spoon	Viscosity measured after three stirs with spoon	thin – thick	Viskositet med ske	√	
Flow from spoon	Continuous flow from spoon	a little – a lot	Sammenhængende flydning fra ske	√	
Meta-descriptor			*Metadeskriptor*		
Creaminess	Perceived creaminess of the sample evaluated in the mouth	a little – a lot	Cremethed	√	√

[*] Pour 0.5 L yoghurt (Jersey 0.1% fat, Thiese Dairy, Denmark) in a dish covered with aluminium foil. Make a number of small holes in the foil and place 3 fried lamb chops on the foil. Wrap close and tight with ceran wrap and leave overnight in refrigerator at 5°C.
[a] In Danish, no word for flavour exists. In Danish taste or "smag" covers both flavour and taste.

Digital images of dairy product surfaces

Camera and set up

The camera used was a Nikon WC-E80, which is a standard three-channel digital camera (RGB), with wide-angle converter, 5.0 mega pixels and 8 × zoom (NIKON CORPORATION, Tokyo, Japan). The digital colour images were RAW (12-bit) of the size 2560 × 1920 pixels. The photography was performed in a dark room. A 15 inch monitor was connected to the camera to ensure that the focus of the image was optimal.

The samples were placed on a baseboard for copy stands. The camera was located on a one meter column (Kaiser Fototechnik GmbH & Co. KG, Germany). Two copy arms with copy lighting units were placed in an angle of 73.3 degrees on each side of the camera (figure 1). The bulbs were Osram, DULUX®S G23, 11W/11-860 850 lumen (LUMILUX, Daylight, Italy). Diffusion foil (Diffusionsfolie 5939, Kaiser Fototechnik) was placed in the light path. No alterations were made in the camera settings between sessions. A Petri dish containing the sample was placed against a grey card with a reference grey colour (The Jessop Group Ltd., Leicester, England). This ensured the same position of the samples in all images and that potential changes in colour between images could be observed.

The dairy samples were prepared by pouring the sample into a Petri dish, tapping the Petri dish lightly ensuring an even spread and then scraping extra sample off in order to obtain a surface with the same height. Three Petri dishes were prepared for all the different samples and three pictures were taken of each Petri dish after rotating it 120 degrees clockwise each time, giving a total of nine images of each sample. Figure 2 shows examples of the obtained images before and after image pre-processing. Image capture of cream cheeses was more strictly controlled as the cheeses with high fat content had a more unstable structure, once ruptured by spreading. Therefore, the first image was taken exactly 1 minute and 10 seconds after surface scrape and the following two images were taken 40 seconds after each other. For the yoghurt experiment the lower part of a Petri dish with a diameter of 8.8 cm was used whereas for the cream cheese it was upper part of a Petri dish with a diameter of 9.2 cm.

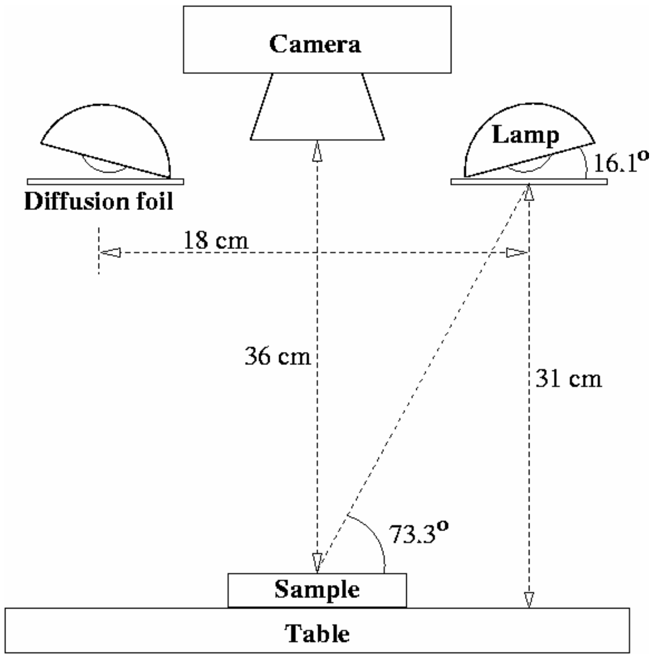

Figure 1. The set up of camera and the two copy arms with lighting units in proportion to the dairy sample.

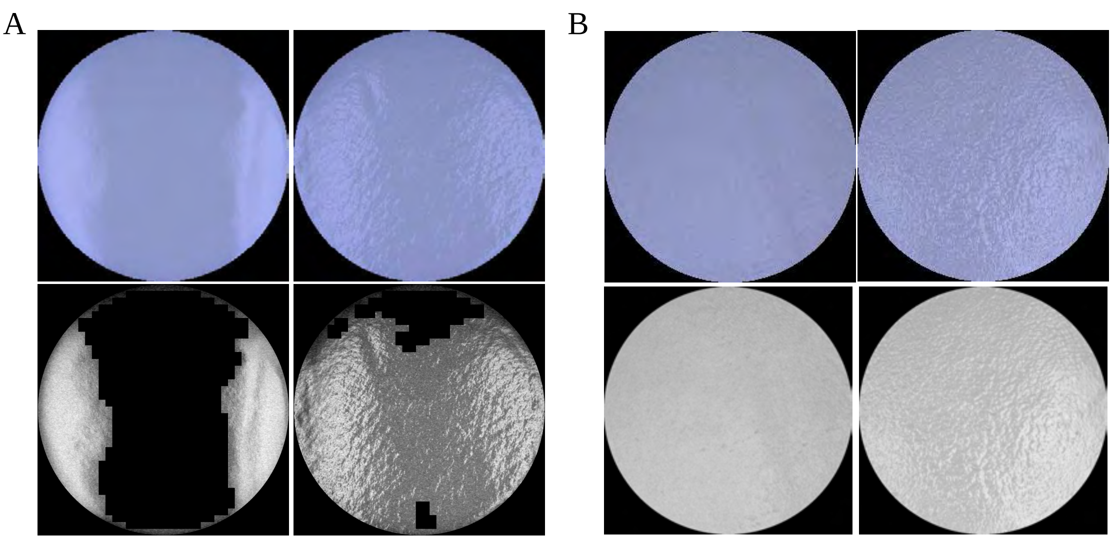

Figure 2. A: Left side images: Skim milk yoghurt without addition of protein. Right side images: Skim milk yoghurt added commercial whey protein concentrate in the highest level. B: Left side images: Cream cheese with 3% fat content, high salt concentration and low pH value. Right side images: Cream cheese with 9% fat content, high salt concentration and high pH value. The upper images are before pre-processing and the lower are after pre-processing.

Image analysis

The images of the dairy products surface was analysed using the Angle Measure Technique (AMT). The used program algorithm is accessible in a Graphical User Interface written in MATLAB 6.5 (The Mathworks, Natick, MA) available on the website http://www.models.kvl.dk. Before image analysis some pre-processing was necessary, also performed in MATLAB. All additional background around the Petri dish was removed in all the images reducing the image size (yoghurt images: 1481 $\times$ 1481 pixels and cream cheese: 1419 $\times$ 1419 pixels (see figure 2)). The images where subsequently filtered for local noise (spatial arithmetic mean filter) and increasing contrast. For yoghurt images, dark uniform regions in the centre of the images was also removed by excluding regions of size 40 $\times$ 40 with a variance lower than 40, thus only including and analysing regions with some surface structure. Figure 2 illustrates the consequence of the pre-processing on selected images of both dairy products. Stratified random start points at a level of 2% were selected prior to unfolding of images. The images were unfolded by horizontally ’snake’ and on all image channels the AMT-linear variant of the algorithm was applied. Detailed explanation of AMT is given in the accompanying manual on the website.

Particle size distribution

The particle size distribution of the cream cheeses was measured by low-angle laser light scattering using a Malvern Mastersizer Microplus and Malvern Small Volume Sampler Presentation Unit, Model DIF 2000 (Malvern Instruments Ltd., Malvern, UK). Cheese was added directly to the Small Volume Sampler Presentation Unit until an obscuration of 20-22% was achieved. The measurements were performed in triplicate. Volume fractions were recorded in 61 intervals from [0.05 μm; 0.06 μm] to [477.01 μm; 555.71 μm].

Data analysis

Multivariate data analysis (Partial Least Squares Regression (PLSR)) was used to investigate all results: both AMT spectra, sensory data, and relationships between those. Initially ANOVA-PLSR (A-PLSR) was applied to evaluate the effect of experimental design factors on the response variables *i.e.* AMT spectra and sensory descriptors (Martens & Martens, 1986; 2001). The method avoids multicollinearity problems by modeling latent variables representing the main variance common for the variables. It is used as a graphical alternative to ANOVA. The AMT spectra from all three colour channels (red, green and blue) were tested, and the channel providing the best result was applied during additional analyses (blue channel for yoghurts and green channel for cream cheese). For sensory data, initially, univariate analysis of variance (ANOVA) was applied to analyse the sensory data. Mixed model ANOVA for individual descriptors was performed with products as fixed factors and panellists as random factors. This method is commonly applied for data from descriptive analysis (Næs & Langsrud, 1998). For descriptors with non-significant Product X Panellist interaction effects, interactions were omitted in a new analysis. Non-significant descriptors were omitted from further analysis. Least significant differences at 5% level (LSD 5%) were estimated based on Mean Square Error. Mean ratings over panelists from each replicate was used for ANOVA-PLSR. For multivariate analyses cross-validation was performed, using segments of 9 images from same sample for AMT spectra, and sensory replicates for sensory data (Martens & Næs, 1989). Jack-knifing served as the validation tool for all multivariate analysis, comparing the perturbed model parameter estimates from cross-validation with the estimates for the full model (Martens & Martens, 2000). For analysis of relationships between surface structure and sensory data, means over the nine extracted AMT spectra from each sample were taken. Afterwards a model on two thirds of the sample set (selected randomly) was tested against one third of the samples (cross-validation). A PLS2 analysis gave an initial overview of the relationships to all sensory descriptors. However, to get the exact result for each descriptor, PLS1 analyses were performed subsequently. All multivariate analyses were made using the Unscrambler 9.1 software (Camo Process AS, Oslo, Norway).

Results and discussion

AMT-spectra

Score plots from A-PLSR on AMT spectra from the yoghurt and cream cheese images are shown in figure 3. Yoghurt samples are coded as a function of added protein to the sample (figure 3a). A systematic effect of fat content, protein type and protein level was observed, indicating that the experimental design provided differences in image surface structure and extracted AMT spectra that can be modelled. Still, perturbed model parameter estimates from Jack-knife cross validation showed that there was not a complete separation of all 25 yoghurts. Roughly speaking, protein level is described in the first Latent variable (different symbols in figure 3a)). Protein type and fat levels are separated in a combination of latent variable 1 and 2, Yoghurts with added skim milk powder (S) or microparticulated milk protein (M) are grouped to the upper right, while samples added high viscosity producing whey protein (V) and commercial whey protein (C) concentrates in the highest level are grouped at the bottom of the plot, except for the yoghurt containing 1.5% fat and microparticulated protein added in the highest concentration (Y1-M-4). Yoghurts added commercial and high viscosity milk protein preparation were grouped in three according to protein level, with the lowest addition at the top and the highest concentration at the bottom of the plot.

The A-PLSR plot of the cream cheese images AMT spectra are coded with respect to the fat content of the samples (figure 3b). Again, the effect of the experimental design is evident, although complete separation of all 18 different cream cheeses was not obtained. For the three lower fat levels, a high pH changes the properties of the spectre, so the samples are grouped with samples with a higher fat level. By contrast, a combination of low pH and low salt content make samples resemble those with a lower fat content. Thus, sample D-F0-S2-p2, with the lowest fat content (0% fat), high salt content (0.9% salt) and high pH value (5.0), is located close to sample M-F9-S1-p1, with the highest fat content (9% fat), low salt content (0.4% salt) and low pH value (4.4).

In both product categories replicate samples are closely located together, in the case of yoghurt the sample 1, 2, 3, and 4 are located close to the centre of the figure whereas for the cream cheese samples Q and R are located to the left and the samples S and T are at the bottom right, respectively. Perturbed model parameter estimates of scores showed high overlap between replicate samples.

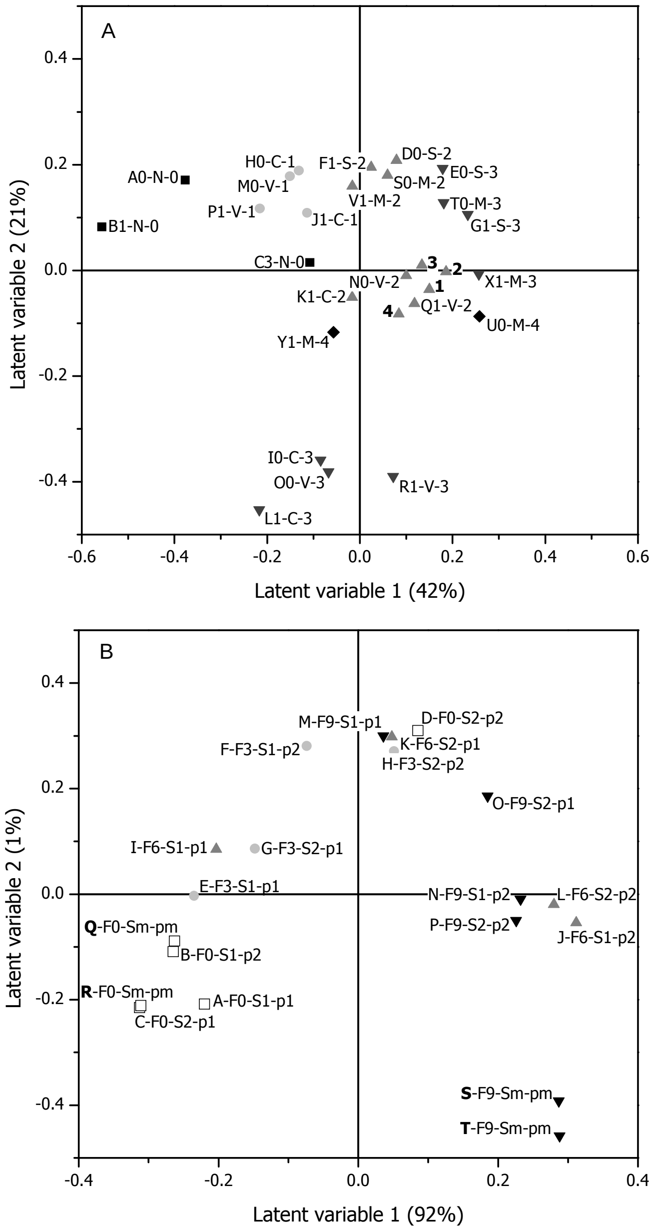

Figure 3. Score plot from A-PLSR performed on the dairy products A: Yoghurt samples are separated after protein content and the variation in the images can be explained using two latent variables. B: Cream cheese samples are separated after fat content and the variation can be explained using 2 latent variables. Refer to table 1 and 2 for product abbreviations

Descriptive analysis

ANOVA on individual descriptors showed that only a few sensory descriptors did not vary significantly over the samples. Scrutiny of results from ANOVA and A-PLSR showed that for both product categories each individual product possessed unique sensory properties, significantly different from all other. The range of mean ratings for each descriptor, together with LSD 5% values are shown in table 4. Perturbed model parameters for A-PLSR showed that for yoghurts product Y1-M-4 varied considerably over the three batch replicates.

Table 5. Mean range of markings for all products (over panellists and replicates) and the Least Significant Differences (LSD) values (p<0.05) for all products and descriptors.

Descriptors	Yoghurt		Cream Cheese	
	Mean range	LSD (5%)	Mean range	LSD (5%)
Aroma				
Tomato smell	[3.49 ; 4.99]	-		
Lamb smell	[2.37 ; 5.63]	0.80		
Cream smell	[1.97 ; 3.35]	0.58	[2.63 ; 8.81]	1.04
Buttermilk smell	[3.43 ; 5.31]	-		
Flour smell	[1.77 ; 3.37]	0.73		
Acidic smell			[5.52 ; 8.98]	0.93
Butter smell			[3.35 ; 9.21]	1.03
Goat smell			[1.37 ; 6.13]	1.00
Old milk smell			[2.08 ; 5.64]	0.88
Non-oral manipulation				
Resistance			[1.78 ; 13.20]	0.78
Appearance				
Whiteness	[9.00 ; 10.42]	0.47	[6.51 ; 11.51]	0.80
Greyness	[1.11 ; 1.87]	-	[0.95 ; 2.51]	0.55
Greenness	[0.67 ; 1.79]	0.41		
Yellowness	[2.09 ; 3.86]	0.51	[1.71 ; 6.48]	0.81
Blueness			[0.82 ; 3.32]	0.67
Glossiness	[5.74 ; 12.05]	0.78	[0.65 ; 12.59]	0.70
Graininess	[1.40 ; 10.13]	0.82		
Grain concentration			[7.34 ; 10.33]	-
Grain size			[2.93 ; 9.76]	1.08
Flavour and taste				
Lamb flavour	[1.95 ; 5.60]	0.74		
Goat flavour			[1.04 ; 5.87]	0.90
Butter flavour	[1.88 ; 5.16]	0.64	[2.18 ; 10.99]	0.92
Cream flavour	[1.89 ; 5.71]	0.72	[2.00 ; 9.94]	0.91
Buttermilk flavour	[3.34 , 6.98]	0.79		
Flour flavour	[1.42 ; 7.67]	0.83		
Sour taste	[4.56 ; 7.42]	0.83	[5.27 ; 11.78]	0.79
Sweet taste	[2.57 ; 6.09]	0.70	[1.23 ; 5.55]	0.68
Salt taste			[3.27 ; 10.48]	0.77
Texture and mouthfeel				
Viscosity	[1.29 ; 12.30]	0.69		
Smoothness	[3.61 ; 11.78]	0.94	[0.93 ; 13.83]	0.63
Firmness			[2.79 ; 12.77]	0.85
Flouriness			[0.55 ; 9.35]	1.65
Chalkiness			[1.26 ; 11.11]	0.77
Graininess			[0.26 ; 13.77]	0.65
Stickiness			[0.78 ; 10.14]	0.75
Meltdown rate	[1.53 ; 10.59]	0.83	[2.14 ; 12.40]	0.74
Astringent	[2.63 ; 5.40]	0.81	[3.74 ; 11.66]	0.92
Fatty after mouthfeel	[1.49 ; 8.01]	0.91		
Dry after mouthfeel	[3.23 ; 7.06]	0.91		
Aftermouthfeel			[5.18 ; 8.91]	-
Non-oral manipulation				
Non-oral viscosity	[0.73 ; 12.42]	0.78		
Graininess on lid	[2.48 ; 12.65]	0.98		
Viscosity with spoon	[1.24 ; 12.61]	0.68		
Flow from spoon	[1.84 ; 14.18]	1.00		
Meta-descriptor				
Creaminess	[1.76 ; 11.12]	0.93	[0.89 ; 12.41]	0.79

Prediction of sensory properties from global image features

Yoghurt

The yoghurt data was analysed with Partial Least Squared Regression (PLSR) by regressing the separately obtained sensory descriptors (Y-variables) on the AMT spectra (X-variables) using test set cross-validation (leaving one third of samples out). The correlation loadings plot from this initial PLS2 analysis displays the descriptors and correlations between these descriptors (see figure 4b). The total explained variance in Y is 52% for the two significant latent variables (39 and 13%). The main variation in the first latent variable use 47% of the variation in the image analysis data to explain 39% of the variation in the sensory data mainly relating to particle size and viscosity. Samples in the left side of the first latent variable had high ratings in a number of texture-related descriptors (*Graininess*, *Graininess on lid*, *Non-oral viscosity*, *Viscosity with spoon*, *Viscosity* and *Meltdown rate*). In the digital images this corresponds to relatively large changes at a small scale, i.e. the intensity (the blue channel in this case) varies considerably over the distance of a low number of pixels (1 to approximately 60 – corresponding to 0.06 – 3.57mm). By contrast, samples in the right side of first latent variable received high ratings in the some other textural descriptors (*Glossiness*, *Smoothness* and *Flow from spoon*). The images of these samples have low variation at the small scale and higher variance at larger scales (starting from approximately 75 to 250 pixels distance – 4.46 to 14.85mm). Some of these descriptors do not relate to appearance (*Viscosity*, *Meltdown rate* and *Smoothness*), but rather to oral perception. The high covariance with visual texture descriptors allows fairly good prediction of these descriptors. The second latent variable uses 31% of the variation in the image analysis to explain 13% of the variation in the sensory analysis. *Creaminess* is not predicted well. Thus, creaminess does not only depend on structural properties reflected in the images, but also on other factors, such as flavour, that are not reflected in the global features extracted from the images. The score plot from the PLS2 analysis show a U-shaped sample spreading (figure 4a). In the upper right corner all the samples without addition of protein (N) are

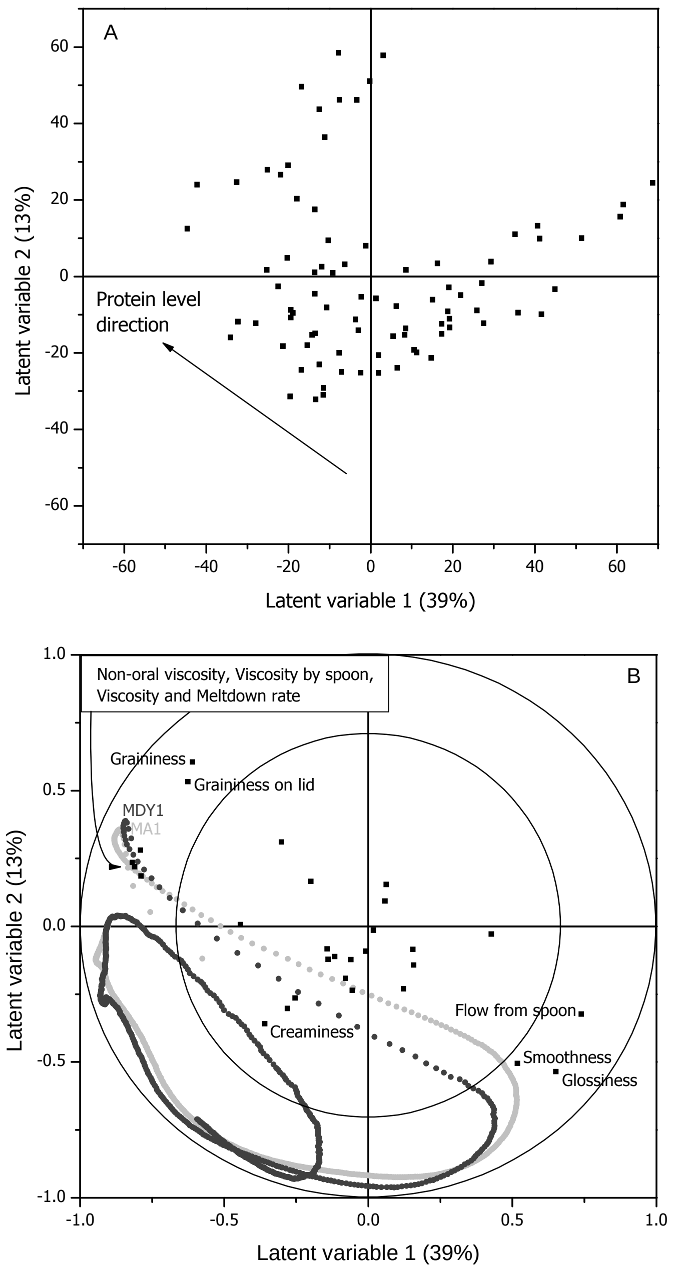

Figure 4. PLSR-modelling of yoghurt data using cross validation. A: Score plot from PLS2 model. Latent variable 1 and 2 separates different levels of protein addition. B: PLSR correlation loadings for the two first dimensions. • Mean angle for increasing scale (MA1), • Mean difference in Y for increasing scale (MDY1) and ▪ Sensory descriptors. The inner and outer circles represent 50% and 100% explained variance, respectively.

located. The samples containing skim milk powder (S) are mainly located in the base of the U-shape whereas the commercial (C), high viscosity producing (V) and microparticulated milk protein preparation (M) are grouped together at the left side of the U-shape. As indicated in figure 4 there is an increase in the protein levels from the right side of the U-shaped plot to the left. Comparing the score and loading plots it is evident that low *Viscosity*, *Glossiness* and *Smoothness* related to samples with lower total protein, while the samples with the high concentration of protein were *Grainy*, *Viscous* and had a slow *Meltdown rate*.

Prediction of individual descriptors from the AMT spectra was performed using PLS1 regression (table 5). The texture-related descriptors have a correlation ≥ 0.83 and a RMSECV ≤ 1.68 except for low prediction of *Creaminess* (correlation = 0.52 and RMSECV = 1.97). Comparing these results with the uncertainty estimates from the sensory analysis (see table 4, LSD (5%)) the prediction error for the best predicted descriptors from the image analysis are in the same range. The next step was to estimate which loading weights (by PLS1 analyses) positively correlate to the different descriptors (table 6). Loading weights show how much each X-variable contribute to explaining the response variation along each model latent variable and hence can be used to find the relationship between the X- and Y-variables. It can be seen that *Glossiness*, *Smoothness* and *Flow from spoon* are correlated both for Mean Angle (MA) and Mean Difference in Y (MDY) when looking at the first two latent variables. *Graininess* and *Grainy on lid* relates at the first and second latent variable but as the only descriptor *Graininess on lid* also have a third latent variable. *Viscosity*, *Meltdown rate*, *Non-oral viscosity* and *Viscosity by spoon* have some similarities to *Graininess* and *Graininess on lid*, although only on the first latent variable.

Table 6. PLS1-modeling of the yoghurt image analysis results against those of the sensory descriptors which could be explained.

Descriptors	AMT						
	Explained X variance (%)	Explained Y variance (%)	Slope	Offset	Correlation	RMSEP	#PLS components
Appearance							
Glossiness	36,42	61,9	0.85	1.53	0.86	1.00	2
Graininess	32,46	65,6	0.90	0.58	0.90	1.06	2
Texture and mouthfeel							
Viscosity	50,28	58,12	0.72	2.06	0.89	1.30	2
Smoothness	31	51	0.59	3.51	0.70	1.62	1
Meltdown rate	51,27	54,11	0.66	2.27	0.87	1.29	2
Non-oral manipulation							
Non-oral viscosity	49,29	57,11	0.70	2.20	0.89	1.47	2
Graininess on lid	36,43,7	57,7,1	0.84	0.66	0.88	1.36	3
Viscosity with spoon	50,28	60,12	0.75	2.03	0.90	1.25	2
Flow from spoon	47,31	54,12	0.70	2.11	0.83	1.68	2
Meta-descriptor							
Creaminess	53	28	0.28	5.33	0.52	1.97	1

Table 7. Positively correlated X-loading weights for the explained sensory descriptors for the yoghurt images, MA – Mean angle and MDY – Mean difference in Y.

Descriptors	Component number					
	PC1		PC2		PC3	
	S_{MA}	S_{MDY}	S_{MA}	S_{MDY}	S_{MA}	S_{MDY}
Appearance						
Glossiness	71-265	28-122, 527-684	60-740	24-412, 479-740		
Graininess	1-62, 415-740	1-23, 196-489	-	-		
Texture and mouthfeel						
Viscosity	1-63, 279-740	1-24, 127-530	1-56	1-20		
Smoothness	78-307	31-149, 511-740				
Meltdown rate	1-65, 274-740	1-25, 132-530	1-65	1-25, 425-470		
Non-oral manipulation						
Non-oral viscosity	1-60, 294-740	1-25, 131-516	1-60	1-25		
Graininess on lid	1-62, 375-740	1-23, 180-490	-	-	1-27, 200-393	1-14, 95-196, 448-521
Viscosity with spoon	1-63, 282-740	1-24, 130-521	1-58	1-24		
Flow from spoon	72-208	29-100	66-545	25-317, 481-740		
Meta-descriptor						
Creaminess	1-36, 158-740	72-740				

Addition of protein increased *Graininess* and *Viscosity*. As reported in earlier studies, creaminess relates both to the textural properties viscosity and smoothness (Guinard & Mazzucchelli, 1996; Kokini & Cussler, 1983; Richardson *et al.*, 1993) but in some product categories it may also depend on flavour (Kilcast & Clegg, 2002; Richardson-Harman *et al.*, 2000). However, no direct information about flavour can be extracted from images, explaining why *Creaminess* can not be predicted well from image analysis in the case of yoghurt.

Cream cheese

Partial Least Squared Regression (PLS2) was performed using test set validation on the data obtained from the surface images and sensory data (see figure 5). The total explained variance in Y is 69% for the two significant latent variables (66 and 3%). The first latent variable uses 98% of the variation in the AMT spectra from the images to explain 66% of the variation in the sensory data relating to nearly all descriptors. At the left side of the first latent variable, the largest contrast difference (MA and MDY) are seen at almost all scales for a number of highly correlated sensory descriptors (*Butter smell*, *Cream smell, Sweet taste*, *Butter flavour*, *Cream flavour*, *Glossiness*, *Meltdown rate*, *Yellowness*, *Smoothness* and *Creaminess*). The samples relating to the above mentioned descriptors exhibit more contrast complexity (larger Mean Angle and Mean Difference in Y) over the whole AMT spectrum. The right side of the plot contains all the descriptors that relate to the samples with the lowest change in contrast in all parts of the scale. The descriptors are

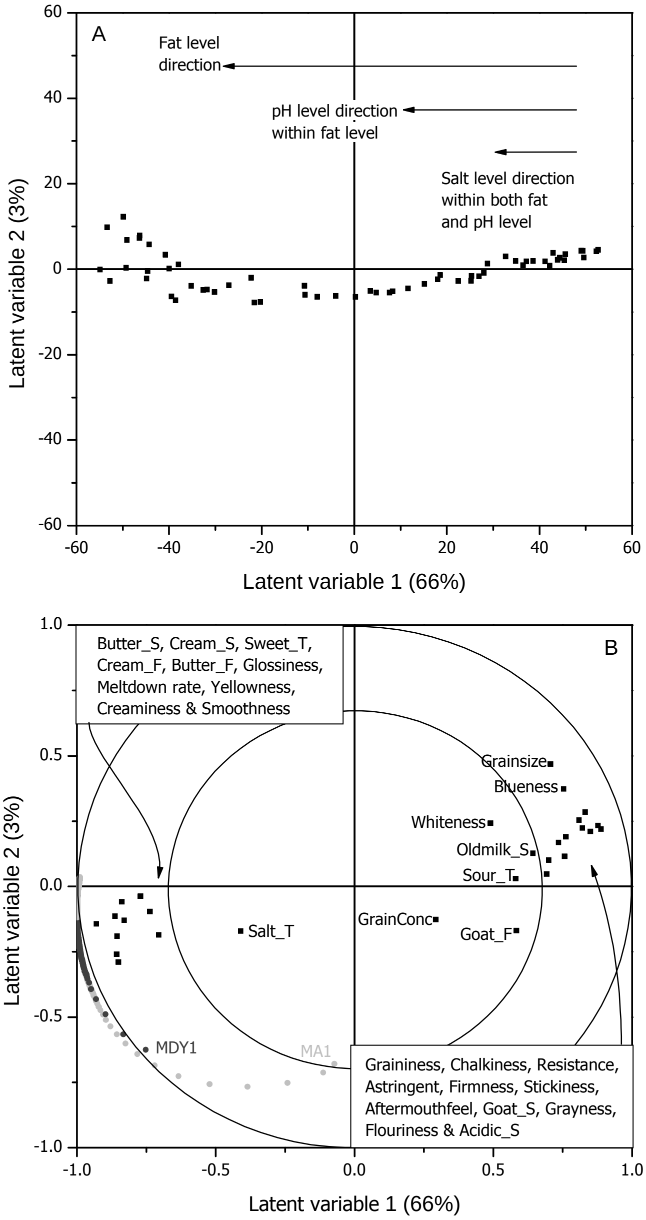

Figure 5. PLSR-modeling of the cream cheese data using test set validation. A: Score plot from PLS2 model. Latent variable 1 separates fat levels and within this separation a difference relating to the pH value. B: PLSR correlation loadings for the two first latent variables. • Mean angle for increasing scale (MA1), • Mean difference in Y for increasing scale (MDY1) and ▪ Sensory descriptors where S = smell, T = taste and F = flavour. The inner and outer circles represent 50% and 100% explained variance, respectively.

Graininess, *Acidic smell*, *Goat smell*, *Blueness*, *Greyness*, *Grain size*, *Resistance*, *Astringent*, *Flouriness*, *Chalkiness*, *Stickiness*, *Firmness*, and *Aftermouthfeel*. The main separation was observed in first latent variable, the second latent variable only uses 2% of the variation in the AMT spectra from the images to explain 3% of the variation in the sensory data. The span of the samples in the score plot is primarily related to the first direction. The main separation is between the cream cheeses' fat content. Furthermore, within this grouping, first of all the increase in pH value and to some degree an increase in salt content changes the samples towards the same properties as those with a higher fat content. Relating the score and loading plots shows that for cream cheese an increase in fat content, as well as pH value, gives more *Glossiness* and *Yellowness* which are visual descriptors closely correlated to both textural descriptors (*Smoothness*, *Meltdown rate*) and smell/taste descriptors (*Butter smell*, *Cream smell*, *Butter flavour*, *Cream flavour* and *Sweet taste*). The results of the PLS1 regression on the cream cheese are listed in table 7. The descriptors of interest have a correlation ≥ 0.74 and a RMSEP ≤ 2.80. When comparing with the panellist error from the sensory analysis (see table 4) the prediction error for the predicted descriptors from the image analysis are acceptable, also for *Creaminess*. The loading weights estimated by the PLS1 analyses can be seen in table 8. The table indicates that for the first latent variable, which explains most of the variation, *Butter smell*, *Cream smell*, *Butter flavor*, *Cream flavor* and *Sweet taste*, are closely correlated to *Yellowness, Glossiness*, *Smoothness, Meltdown rate* and *Creaminess* represented by the same X-loadings weights. The descriptors *Acidic smell*, *Greyness*, *Stickiness*, *Flouriness*, *Astringent*, *Aftermouthfeel*, *Firmness*, *Graininess*, *Chalkiness*, *Resistance*, *Blueness*, *Goat smell*, *Old milk smell,* and *Grain size* are negatively correlated to all of the AMT spectra in the first latent variable.

Table 8. PLS1-modeling of the image analysis results against those of the sensory descriptors which could be explained.

Descriptors	AMT						
	Explained X variance (%)	Explained Y variance (%)	Slope	Offset	Correlation	RMSEP	#PLS components
Aroma							
Acidic smell	98	45	0.47	3.74	0.74	0.70	1*
Butter smell	98	56	0.58	2.72	0.81	1.13	1
Cream smell	98	69	0.68	1.93	0.86	0.97	1
Goat smell	98, 2, 0	53, 3, 7	0.58	1.36	0.82	0.86	3
Old milk smell	98, 1, 1	42, 18, 6	0.63	1.25	0.84	0.50	3
Non-oral manipulation							
Resistance	98, 2	77,2	0.79	1.19	0.89	1.62	2*
Appearance							
Blueness	98, 2, 0	57, 11, 8	0.72	0.48	0.89	0.36	3
Greyness	98	57	0.55	0.76	0.76	0.29	1*
Yellowness	98, 2, 1	52, 5, 7	0.55	1.85	0.76	0.87	3*
Glossiness	98	84	0.84	1.08	0.96	1.24	1*
Grain size	98, 2, 0	52, 19, 14	0.80	1.11	0.90	0.95	3*
Flavour and taste							
Butter flavour	98	66	0.67	2.07	0.87	1.33	1
Cream flavour	98	73	0.72	1.78	0.88	1.26	1*
Sweet taste	98	51	0.53	1.59	0.78	0.75	1*
Texture and mouthfeel							
Firmness	98, 2	79, 2	0.80	1.33	0.90	1.41	2*
Smoothness	98, 2	72, 5	0.77	2.35	0.87	2.41	2*
Meltdown rate	98	73	0.70	2.34	0.86	1.76	1*
Astringent	98, 2	63, 2	0.71	2.08	0.88	1.42	2*
Flouriness	98	46	0.49	2.24	0.75	1.81	1
Chalkiness	98, 2	64, 3	0.69	1.27	0.84	1.75	2*
Graininess	98, 2	69, 5	0.73	1.06	0.84	2.80	2*
Stickiness	98	71	0.70	1.44	0.87	1.69	1*
Aftermouthfeel	98, 2	57, 3	0.62	2.64	0.79	0.67	2
Meta-descriptor							
Creaminess	98, 2	71, 4	0.78	1.93	0.89	2.00	2

*The actual decrease in RMSEP continued further than the chosen PLS components, however the loadings became unstable when using more then the chosen PLS components.

Table 9. Positively correlated X-loading weights for the explained sensory descriptors for the cream cheese images, MA – Mean angle and MDY – Mean difference in Y.

Descriptors	Component number					
	PC1		PC2		PC3	
	S_{MA}	S_{MDY}	S_{MA}	S_{MDY}	S_{MA}	S_{MDY}
Aroma						
Acidic smell	-	-				
Butter smell	4-709	1-709				
Cream smell	4-709	1-709				
Goat smell	-	-	-	405-709	8-260	1-115
Old milk smell	-	-	36-139	7-42	1-221	1-113
Non-oral manipulation						
Resistance	-	-	594-709	294-709		
Appearance						
Blueness	-	-	578-709	279-709	12-249	5-97
Greyness	-	-				
Yellowness	1-709	1-709	1-101	1-38	624-709	480-709
Glossiness	4-709	1-709				
Grain size	-	-	524-709	280-709	14-269	2-119
Flavour and taste						
Butter flavour	4-709	1-709				
Cream flavour	4-709	1-709				
Sweet taste	4-709	1-709				
Texture and mouthfeel						
Firmness	-	-	575-709	266-709		
Smoothness	2-709	1-709	1-266	1-130		
Meltdown rate	4-709	1-709				
Astringent	-	-	484-709	205-709		
Flouriness	-	-				
Chalkiness	-	-	594-709	285-709		
Graininess	-	-	614-709	288-709		
Stickiness	-	-				
Aftermouthfeel	-	-	624-709	266-709		
Meta-descriptor						
Creaminess	3-709	1-709	1-336	1-142		

The particle size distributions of the cream cheeses (see figure 6), largely showed that the non-fat samples exhibited a bimodal size distribution (it is thus less meaningful to characterize the size distribution by an average value). A clear inverse relationship is also seen between fat content and particle size. An increase in the pH value decreases the amount of largest particles, but does not shift the histogram towards overall smaller particles as is the case when increasing salt content. Relating the surface images and the results from the Mastersizer shows that the samples containing 9% fat (except the one with the lowest pH value and salt content), all samples with 6% fat and high pH value and samples with 0 and 3% fat with the highest pH value and highest salt content all contained particles in the range 0.8 to 19µm and images with high contrast complexity. The rest of the samples with 0 and 3% fat and the samples with 6% fat at low pH and low salt mainly contained particles ranging from 66 to 410µm and the surface images all exhibited low contrast complexity.

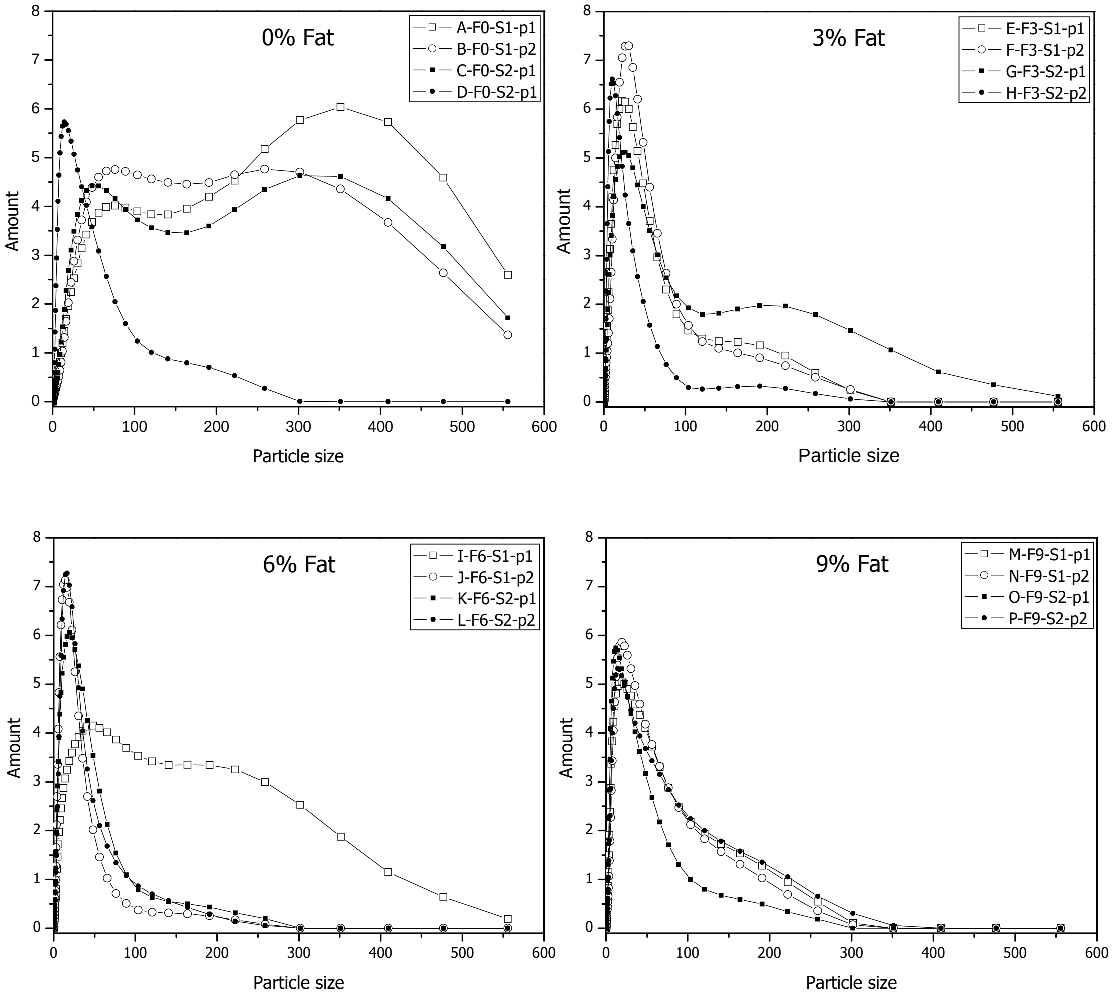

Figure 6. Distribution of particles size for the cream cheeses containing different levels of fat. For each fat level the effect of pH and salt content can be seen.

These results indicate that decreasing the fat content in cream cheese increases the amount of large particles which both affects the product appearance and the textual sensation, as these samples for example are more *Grey*, *Grainy* and *Firm*. However, the concentration of large particles can be affected both by pH value and salt content, in that an increase in both will reduce the number of large particles, due to a reduced aggregation of acid milk gel particles. This reduction in the amount of large particles changes the appearance of a low fat cream cheese towards the higher fat containing cream cheeses, as these samples are more *Yellow*, *Glossy*, *Smooth* and *Creamy*. The fairly good prediction of *Creaminess* in cream cheese indicates that in this study it is related to textural properties in a more straightforward manner than in the case of yoghurt.

Conclusion

The results indicate that all of the design variables had an effect on the dairy products visual appearance. For yoghurt addition of protein was the most important factor separating the samples, followed by protein type and fat content. For the cream cheeses, the fat content had the highest impact, followed by the pH value and the salt concentration. For both dairy product categories the strong correlation between appearance and structural properties made it possible to relate image features to perceived textural properties and for the cream cheese even smell, taste and flavour could be distinguished. For both products the prediction error for the best predicted descriptors from the image analysis was approximately the same as the panellist error in the sensory analysis.

Prediction of perceived creaminess was better in cream cheese than in yoghurt. This indicates that the perception of creaminess in the chosen set of low fat cream cheese was more straightforwardly related to textural properties, whereas the perception of creaminess in the chosen set of yoghurts requires some specific texture and flavour properties.

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Prediction of sensory properties of semi-solid dairy products from confocal laser scanning micrographs using global feature extraction and multivariate regression techniques

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Abstract

Studies linking the microstructure of dairy products to sensory properties are mostly qualitative in nature. In the present work we have related sensory data to confocal laser scanning images of yoghurt and cream cheese using feature extraction techniques (Angle Measure Technique and Fourier transform magnitude spectra) and PLS regression. Application of AMT and FFT made it possible to discriminate between microstructures and find some of the same correlations seen in the sensory analysis alone. In particular, a correlation between yoghurt microstructure and the sensory property creaminess was found.

Keywords

Yoghurt, Cream cheese, Sensory descriptive analysis, Image analysis

Introduction

Confocal laser scanning microscopy (CLSM) has proven an excellent tool for studying food structure (Blonk and van Aalst, 1993). It is suitable for examining the general

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microstructure of milk gels, but not the finer details or internal structure of features like casein micelles or protein clusters (Heertje *et al.*, 1985). CLSM has been used extensively to characterize milk gel structures, but mostly qualitatively (Lucey *et al.*, 1998b; Lucey *et al.*, 1998a). Studies relating large data sets, e.g. of sensory data, to CLSM images require image analysis algorithms which extract relevant features. One approach is to measure relevant properties such as mean pore size (Pereira *et al.*, 2006); correlating these properties with sensory data is straight-forward with univariate linear regression. Another way to deal with the issue is to extract global features from the images. This is particularly relevant to near-isotropic image such as CLSM images of milk gels. Image texture methods can be divided in four categories: statistical, structural, model-based and transform-based (Bharati *et al.*, 2004).

Both methods employed in this study belong to the group of transform-based texture methods. Kvaal *et al.* (1998b) compared different multivariate feature extraction methods on images of wheat baguettes with regards to their ability to predict sensory descriptors from a full descriptive analysis. The Angle Measure Technique turned out to be the best in predicting sensory porosity. In addition, the AMT-based models required fewer latent variables, and were thus deemed more robust. In a similar study on mayonnaise, Indahl and Næs (1998) found that the Absolute Difference Spectrum (ABDF) method predicted a subset of five sensory descriptors slightly better than the magnitude spectrum of the Fourier transform, which in turn performed better than, among other, the Angle Measure Technique (although no statistically significant difference between any of the methods considered was found). It was inferred that the Fourier-based and similar feature extraction methods are preferable for images containing periodic phenomena, whereas the AMT spectra seemed to contain more information in the case of more irregular structures (Kvaal *et al.*, 1998a).

The method Angle Measure Technique (AMT) was introduced by Robert Andrle in 1994 as an alternative method to fractal analysis in characterizing the complexity of two-dimensional geomorphic lines (Andrle, 1994). Esbensen *et al.* (1996) investigated the use of AMT on other applications than geological images. In contrast to Andrle who analyzed an image of a curve, Esbensen *et al.* analyzed an unfolded (vectorized) image. AMT has since been evaluated on different food products. Scanning electron microscopy images of dressing systems were examined by Egelandsdal *et al.* (1999) and on powders (Huang and Esbensen, 2000; 2001).

The Fourier transform is widely used in image processing and analysis. Discrete Fourier Transforms (DFT) on isotropic images yields a matrix of the same dimensions as the image itself. For isotropic image the DFT array is approximately rotationally symmetric around the zero-frequency (centre of the array), this means that the features are (to within the approximation) only located in one dimension. This dimension is the radial axis of the polar coordinate system with centre equal to the zero-frequency point. The projection of the DFT array onto this axis is called the radial average:

$$R(r) = \frac{1}{|C_r|} \sum^{(i,j)\in C_r} F(i,j)$$

where C_r is the set of points belonging to the digitized circle of radius r and F may be the real, imaginary or the amplitude of the elements in the DFT array. Similarly, an angular average may be defined as:

$$A(\theta) = \frac{1}{|L_\theta|} \sum^{(i,j)\in L_\theta} F(i,j)$$

where L_θ is the set of points belonging to the digitized line with angle θ to the abscissa. Figure 1 shows the principle of radial averages graphically.

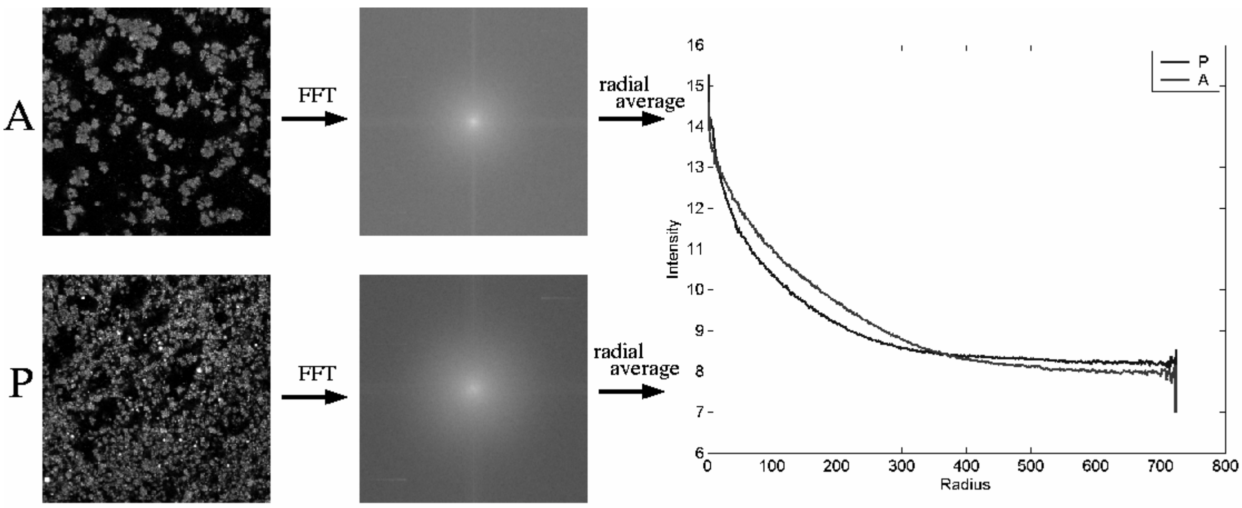

Figure 1. Two CLSM images A and P, their Fourier transform magnitude, and derived radial averages.

Although the angular average is in principle negligible for isotropic images, in reality there is always some information to gain from it, since nothing is perfectly isotropic.

Materials and methods

A total of 25 batches of stirred yoghurt (Janhøj *et al.*, 2006b) and 20 batches of cream cheese were evaluated (Janhøj *et al.*, 2006a). Design variables for the yoghurt study were fat level, protein type and protein level (see Table 1).

Table 1. The 25 analysed yoghurts abbreviations and composition. The different contents of fat (0, 1, 3) and protein (0, 1, 2, 3, 4) added and a short description of these proteins (N, S, C, V, M).

Product abbreviations	Fat content (%)			Added protein type	Total protein level (w/w%)				
	0	1	3	(N, S, C, V, M)	0	1	2	3	4
A0-N-0	0.3			None (N)	3.3				
B1-N-0		1.5			3.3				
C3-N-0			3.5		3.3				
D0-S-2	0.3			Skimmed milk powder (S)			4.8		
E0-S-3	0.3							5.4	
F1-S-2		1.5					4.8		
G1-S-3		1.5						5.4	
H0-C-1	0.3			Commercial milk protein preparation (C)		4.2			
REF0-C-2*	0.3						4.8		
I0-C-3	0.3							5.4	
J1-C-1		1.5				4.2			
K1-C-2		1.5					4.8		
L1-C-3		1.5						5.4	
M0-V-1	0.3			High viscosity milk protein preparation (V)		4.2			
N0-V-2	0.3						4.8		
O0-V-3	0.3							5.4	
P1-V-1		1.5				4.2			
Q1-V-2		1.5					4.8		
R1-V-3		1.5						5.4	
S0-M-2	0.3			Microparticulated milk protein preparation (M)			4.8		
T0-M-3	0.3							5.4	
U0-M-4	0.3								6.0
V1-M-2		1.5					4.8		
X1-M-3		1.5						5.4	
Y1-M-4		1.5							6.0

The yoghurt with 0.3% fat added commercial milk protein preparation adjusted to 4.8% total protein was selected as the reference to appear in all 12 sensory sessions. These 12 samples were treated as 4 different products (1, 2, 3 and 4). Due to CLSM equipment failure, only half of the image data set could be used in the yoghurt trial, corresponding to the second half of the second replicate and all of the third replicate.

The cream cheese samples were varied in fat content, pH and salt concentration (see Table 2).

Table 2. The 20 analysed cream cheeses abbreviations and composition. The different contents of fat (0, 3, 6, 9), salt content (1, m, 2) and pH value (1, m, 2).

Product abbreviations	Fat content (%)				Salt content (%)			pH value		
	0	3	6	9	1	m	2	1	m	2
A-F0-S1-p1	0.0				0.4			4.4		
B-F0-S1-p2	0.0				0.4					5.0
C-F0-S2-p1	0.0						0.9	4.4		
D-F0-S2-p2	0.0						0.9			5.0
E-F3-S1-p1		3.0			0.4			4.4		
F-F3-S1-p2		3.0			0.4					5.0
G-F3-S2-p1		3.0					0.9	4.4		
H-F3-S2-p2		3.0					0.9			5.0
I-F6-S1-p1			6.0		0.4			4.4		
J-F6-S1-p2			6.0		0.4					5.0
K-F6-S2-p1			6.0				0.9	4.4		
L-F6-S2-p2			6.0				0.9			5.0
M-F9-S1-p1				9.0	0.4			4.4		
N-F9-S1-p2				9.0	0.4					5.0
O-F9-S2-p1				9.0			0.9	4.4		
P-F9-S2-p2				9.0			0.9			5.0
Q-F0-Sm-pm	0.0					0.65			4.7	
R-F0-Sm-pm	0.0					0.65			4.7	
S-F9-Sm-pm				9.0		0.65			4.7	
T-F9-Sm-pm				9.0		0.65			4.7	

The cream cheeses (Q, R, S, T) with the lowest and highest fat content adjusted to both average salt content and pH-value were selected as the reference to appear twice.

Confocal laser scanning microscopy

Yoghurt

Due to the inherently unstable nature of the sirred yoghurt, sensory evaluations and microscopy were performed on exactly seven day old samples. A Leica TCS SP2 confocal laser scanning system (Leica Microsystems, Mannheim, Germany) fitted with an upright Leica DM RXE microscope and an argon laser with excitation at 488 nm was used. Filter settings for the green channel were at wavelengths emitted at 500-535 nm. The lens was a 20 $\times$ 0.4 Na Dry N-Plan lens.

The hydrophilic protein network in the yoghurt samples were dyed with the fluorescent dye, Oregon Green® 488 (D-6145) from Molecular Probes, Eugene, OR, USA. A solution of dye was made by dissolving 0.0019g dye in 2mL ethanol giving a 0.095% solution.

This was afterwards diluted with ethanol to a 0.0095% solution, which then was filtered using a 20µm micro filter and kept in the dark at 5ºC.

Samples were prepared first by placing a thin layer of dye solution on a covering glass and letting it dry. The sample of yoghurt was then placed on the slide in an amount that could spread and give a relatively big surface. The covering glass was placed on the yoghurt sample and fixed with correction fluid. After drying the sample could be placed in the microscope.

It was attempted to obtain a picture in the same depth of all the samples. Therefore each time a new slide was placed in the microscope, the microscope was re-zeroed. The xz-plan was used to positioning the covering glass at the centre of the screen before starting to focus in the xy-plan. Each image had the format 1024 $\times$ 1024 pixels for 750 $\times$ 750 µm and consisted of an accumulation of four images obtained at the exact same position in the yoghurt. Three slides were prepared for all samples and three pictures were taken of each slide, giving a total of nine images of each yoghurt sample.

Cream cheese

For the cream cheese samples a Leica TCS SP confocal laser scanning system (Leica Microsystems, Mannheim, Germany) fitted with an inverted Leica DM IRBE microscope and an argon/krypton laser with excitation at 488 and 568 nm was used. Filter settings for the green channel were at wavelengths emitted at 500-535 nm. The lens was a 20 $\times$ 0.4 Na Dry N-Plan lens and a 63 $\times$ N-Plan lens.

The cream cheese samples were dyed with the non-specific dye Nile Red (Molecular Probes, Eugene, OR) as well as with Oregon Green® 488 (D-6145). The Nile Red solution was prepared as the Oregon Green mentioned before.

Samples were prepared first by placing a thin layer of an equal mixture of Oregon Green and Nile red on a covering glass and letting it dry. A thin slice of cream cheese sample was then placed on the slide in an amount that gave a relatively big surface.

The covering glass was placed on the sample and fixed with correction fluid. After drying the sample could be placed in the microscope.

Three slides were prepared for all samples and five pictures were taken of each slide, giving a total of 15 images of each cream cheese sample.

Image analysis

Prior to image analysis, pre-processing was necessary. The images were made more equal in local brightness level by local grid adjustment (*xpolyxyfit*), followed by removal of local noise (convolution) and finally magnification of the differences in contrast. Figure 2 shows a images before and after pre-processing.

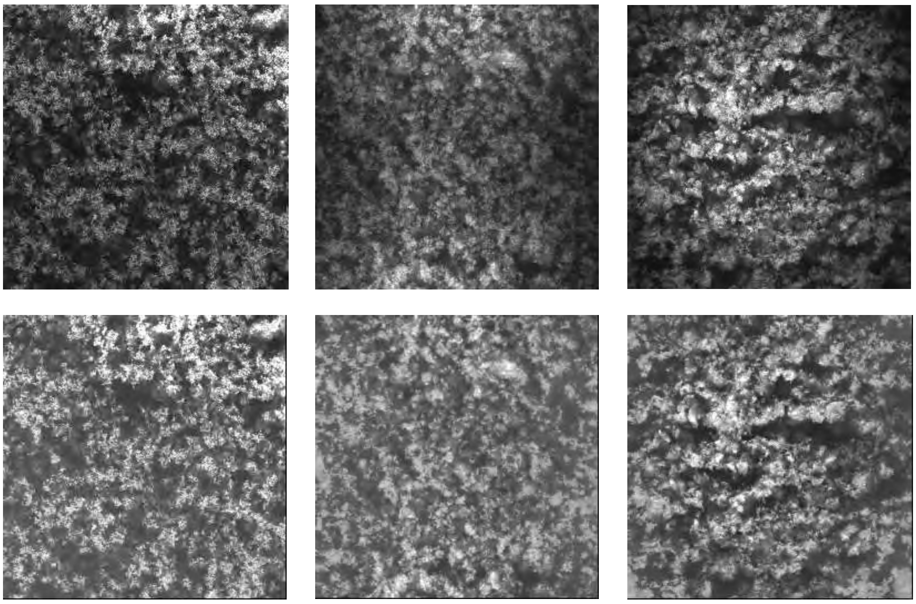

Figure 2. From left to right are shown the microstructure images before (upper) and after pre-processing (lower) from yoghurt A, G and I.

The pre-processed CLSM images of yoghurt were analysed by the Angle Measure Technique (AMT) using the IMAT Toolbox (Laugesen *et al.*, 2005) under MATLAB 6.5 (The MathWorks, Natick, MA). The AMT-linear algorithm was used to analyse the vertically unfolded images ("classic" unfolding). AMT-linear uses a "random" point A and a value *s*, the points B and C are defined as the point at distances *s* on the *x*-axis to the right and left of A, respectively.

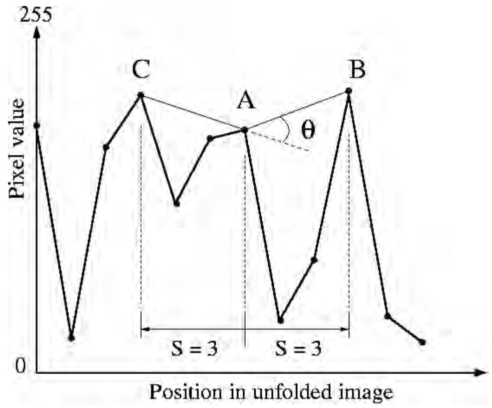

Figure 3. Illustration of the AMT-linear.

When increasing *s*, B and C are simply the next pixels in the unfolded sequence of pixels. The grid sampling was preformed before unfolding with approximately 1% stratified randomly selected samples. This gave a grid of 100 by 100 with one random point within each grid box. The value *s* increased to 1024, the size of the images.

All CLSM images of cream cheese were analyzed by the radial mean of Fast Fourier transform algorithm using the IMAT Toolbox. The log power of the DFT, F=log(|DFT(X)|) was used.

Sensory descriptive analysis

The samples were evaluated sensorially by descriptive analyses using a trained panel. The descriptors, tabulated in Table 1 and Table 4 were developed by consensus, with the exception of the meta-descriptor *creaminess*.

Table 3. Sensory descriptors (yoghurt study) their definitions and original terms in Danish.

Descriptors	Definition (reference material)	Anchor points	Original terms in Danish
Aroma			*Lugt*
Tomato smell	Intensity of tomato aroma (0.3 L yoghurt (Jersey 0.1% fat, Thise Dairy, Denmark) added 5 drops of Heinz ® Tomato Ketchup)	a little – a lot	Lugt af tomat
Lamb smell	Intensity of lamb aroma (see below for detailed procedure*)	a little – a lot	Lugt af lam
Cream smell	Intensity of raw cream aroma (full fat homogenised milk (3.5% fat) and cream (38% fat) in a 1 to 5 mixture)	a little – a lot	Flødelugt
Buttermilk smell	Intensity of buttermilk aroma (Organically produced buttermilk (ArlaFoods, Denmark))	a little – a lot	Kærnemælkslugt
Flour smell	Intensity of flour aroma (0.3 L yoghurt (Jersey 0.1% fat, Thise Dairy, Denmark) added 15 mL wheat flour)	a little – a lot	Melet lugt
Appearance			*Udseende*
Whiteness	Intensity of the colour white	a little – a lot	Hvid farve
Greenness	Intensity of the colour green	a little – a lot	Grøn farve
Greyness	Intensity of the colour grey	a little – a lot	Grå farve
Yellowness	Intensity of the colour yellow	a little – a lot	Gul farve
Glossiness	Degree of yoghurt surface shininess	a little – a lot	Blankhed
Graininess	Degree of yoghurt surface graininess	a little – a lot	Grynethed
Flavour and taste			*Smag*
Lamb flavour	Intensity of lamb flavour (see above)	a little – a lot	Smag af lam
Butter flavour	Intensity of butter flavour (Lumb of organically produced old fashioned churned, salted butter (Lurpak ®, ArlaFoods, Denmark))	a little – a lot	Smag af smør
Cream flavour	Intensity of cream flavour (see above)	a little – a lot	Smag af fløde
Buttermilk flavour	Intensity of buttermilk flavour (see above)	a little – a lot	Smag af kærnemælk
Flour flavour	Intensity of flour flavour (see above)	a little – a lot	Melet smag
Sour taste	Intensity of sour taste	a little – a lot	Sur smag
Sweet taste	Intensity of sweet taste	a little – a lot	Sød smag
Texture and mouthfeel			*Konsistens*
Viscosity	Perceived thickness of the sample evaluated in the mouth	thin – thick	Viskositet
Smoothness	Perceived smoothness of the sample evaluated in the mouth	a little – a lot	Glathed
Meltdown rate	Amount of "work" to break down the bolus	slow – fast	Nedsmeltning
Astringent	Intensity of saliva losing feeling in the mouth – using the tough against the palate or the back of the teeth	a little – a lot	Astringerende
Fatty after mouthfeel	Degree of "fatty" mouth coating after expectoration of the sample	a little – a lot	Fedtet eftermundfylde
Dry after mouthfeel	Degree of mouth dryness after expectoration of the sample	a little – a lot	Tør eftermundfylde
Non-oral manipulation			*Manipulation med ske*
Non-oral viscosity	Rate of a spoon full to blur when it is placed on top of the sample	a little – a lot	Gelstivhed
Graininess on lid	Half a spoon of sample spread on a lid	a little – a lot	Grynethed på låg
Viscosity with spoon	Viscosity measured after three stirs with spoon	thin – thick	Viskositet med ske
Flow from spoon	Continuous flow from spoon	a little – a lot	Sammenhængende flydning fra ske
Metadesciptor			*Metadeskriptor*
Creaminess	Perceived creaminess of the sample evaluated in the mouth	a little – a lot	Cremethed

Table 4. Sensory descriptors (cream cheese study), their definitions and original terms in Danish.

Descriptors	Definition (reference material)	Anchor points	Original term in Danish
Aroma			*Lugt*
Cream smell	Intensity of raw cream aroma (full fat homogenised milk (3.5% fat) and cream (38% fat) in a 1 to 5 mixture)	a little – a lot	Flødelugt
Acidic smell	Intensity of acidic smell when opening the sample	a little – a lot	Syrlig lugt
Butter smell	Intensity of butter flavour (Lump of organically produced old fashioned churned, salted butter (Lurpak ®, ArlaFoods, Denmark))	a little – a lot	Smørlugt
Goat smell	Intensity of goat aroma (goat yoghurt)	a little – a lot	Gedelugt
Old milk smell	Intensity of old milk aroma	a little – a lot	Gammel mælk lugt
Non-oral manipulation			*Manipulation med hånd*
Resistance	Resistance during spread with a knife	low - high	Modstand
Appearance			*Udseende*
Whiteness	Intensity of the colour white	a little – a lot	Hvid farve
Greyness	Intensity of the colour grey	a little – a lot	Grå farve
Yellowness	Intensity of the colour yellow	a little – a lot	Gul farve
Blueness	Intensity of the colour blue	a little – a lot	Blå farve
Glossiness	Degree of surface shininess	a little – a lot	Blankhed
Grain concentration	Evaluation of closeness of grains	a little – a lot	Koncentration af gryn
Grain size	Evaluation of the average size of grains	small – large	Størrelse af gryn
Flavour and taste			*Smag*
Goat flavour	Intensity of goat flavour (see above)	a little – a lot	Smag af ged
Butter flavour	Intensity of butter flavour (Lump of organically produced old fashioned churned, salted butter (Lurpak ®, ArlaFoods, Denmark))	a little – a lot	Smag af smør
Cream flavour	Intensity of cream flavour (see above)	a little – a lot	Smag af fløde
Sour taste	Intensity of sour taste	a little – a lot	Sur smag
Sweet taste	Intensity of sweet taste	a little – a lot	Sød smag
Salt taste	Intensity of salt taste	a little – a lot	Salt smag
Texture and mouthfeel			*Konsistens*
Smoothness	Perceived smoothness of the sample evaluated in the mouth	a little – a lot	Glathed
Firmness	Perceived firmness of the sample evaluated in the mouth	a little – a lot	Fasthed
Flouriness	Intensity of flour aroma (0.3 L yoghurt (Jersey 0.1% fat, Thise Dairy, Denmark) added 15 mL wheat flour)	a little – a lot	Melethed
Chalkiness	Perceived chalkiness of the sample evaluated in the mouth	a little – a lot	Kridtethed
Graininess	Perceived graininess of the sample evaluated in the mouth	a little – a lot	Grynethed
Stickiness	Perceived stickiness of the sample evaluated in the mouth	a little – a lot	Klistrethed
Meltdown rate	Amount of "work" to break down the bolus	slow – fast	Nedsmeltning
Astringent	Intensity of saliva losing feeling in the mouth – using the tough against the palate or the back of the teeth	a little – a lot	Astringerende
Aftermouthfeel	Degree of mouth coating after expectoration of the sample	a little – a lot	Eftermundfylde
Meta-descriptor			*Metadeskriptor*
Creaminess	Perceived creaminess of the sample evaluated in the mouth	a little – a lot	Cremethed

Data analysis

The microstructure of the yoghurts and cream cheese were related to the sensory data by regressing the sensory data matrices on the extracted image feature spectra uning partial least squares regression (PLSR). Averages over the nine images of each yoghurt sample, and 15 images of each cream cheese were used as the independent variable. The PLS models were croo-validated Subsequently a model on a whole sample set (first or third replicate) and tested it against one half the samples (half of the second replicate). Both averaged datasets based on AMT spectra from all samples, and sets from which potential outliers had been removed were analyzed. The cream cheese models were validated by leave-one-out cross validation. Both averaged datasets based on FFT magnitude spectra from all samples and sets based on average samples without potential outliers were tested.

Initally PLS2 analyses (multivariate dependent sensory variable Y) were performed, followed by more accurate PLS1 analyses (univariate dependent sensory variable Y). All multivariate analyses were performed using the Unscrambler 9.1 software (Camo ASA, Trondheim, Norway).

Results and discussion

Microstructure data - Yoghurt

To get a general view of the relation between the data obtained from the microstructure images (X-variables) and sensory data (Y-variables) partial least squares regression (PLSR) was performed using test set validation (see Figure 4).

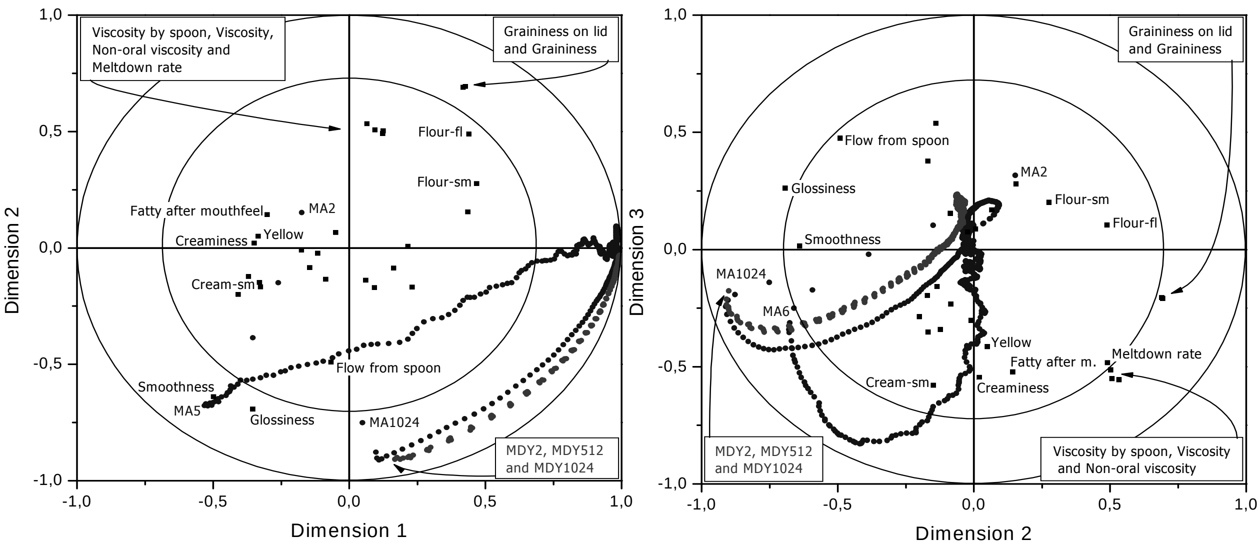

Figure 4. PLS2 modelling results for the first three dimensions. X-var. are the AMT spectra and Y-var. the sensory descriptors. X-var 91.3.4.1 / Y-var 11.28.19.5

The total explained variance in Y is 63% for the fire significant dimensions (11, 28 19 and 5%). The first three dimensions uses 98% of the variation in the AMT spectres to explain 58% of the variation in the sensory data relating to some of the used descriptors. At the lower left corner of the correlation loadings plot of the first and second dimension the contrast difference (MA and MDY) are small scales, corresponding to *glossiness* and *smoothness*. The upper right side of the plot contains all the descriptors that relate to the samples with an over all higher contrast difference at all scales. The descriptors are *graininess* and *graininess on lid*. The plot of the second and third dimension shows that the viscosity related descriptors also can be predicted. In neither plot is c*reaminess* well predicted.

Because of the small number of image samples (due to the equipment failure), the precision of the PLS models was limited. No samples were removed before the models were made as this would change the experimental design completely, even though some samples had an outlier tendency (yoghurt B). The 0.3% fat yoghurt without added protein (yoghurt A) was found not to be an outlier but an extreme sample and removal of this sample did not affect the PLS2 modelling.

Analysis of the AMT spectra includes analysing the X-loading weights estimated by PLS1 regression (see Table 5).

Table 5. PLS1-modeling of the yoghurt image analysis results against those of the sensory descriptors which could be explained. *Use more PLS components but the explained X-varians does not bring any more information then the used components

	Explained X variance (%)	Explained Y variance (%)	Slope	Offset	Correlation	RMSEP	#PLS components
Aroma							
Cream smell	89,8	9,27	0.36	1.26	0.74	0.36	2
Flour smell	91,6	24,8	0.32	1.35	0.55	0.62	2
Appearance							
Yellowness	91,5	7,8	0.25	2.33	0.56	0.63	2
Glossiness	91,5,2,1	15,31,25,4	0.59	4.50	0.80	1.63	4
Graininess	91,5,2	22,27,23	0.57	1.93	0.82	1.72	3
Flavour/Taste							
Flour flavour	91,6,1	21,14,12	0.34	2.10	0.73	1.83	3
Texture/ Mouthfeel							
Viscosity	90,2,5,1	3,62,3,5	0.70	1.48	0.72	2.64	4*
Smoothness	91,6,2	27,26,21	0.59	4.05	0.77	1.85	3
Meltdown rate	91,2	4,55	0.45	2,98	0.61	2.59	2
Fatty after mouthfeel	91,5	10,21	0.39	2.47	0.59	1.54	2
Manipulation with spoon							
Non-oral viscosity	91,2	4,61	0.52	3.01	0.63	3.04	2
Graininess on lid	91,5,2	23,30,23	0.62	3.38	0.85	1.99	3
Metadescriptor							
Creaminess	91,6	17,17	0.41	3.86	0.63	2.00	2

The positively correlated X-loading weights for the sensory descriptors are tabulated in Table 6.

Table 6. Positively correlated X-loading weights for the sensory descriptors, MA – Mean angle and MDY – Mean difference in Y

Descriptors	Component number							
	1		2		3		4	
	MA	MDY	MA	MDY	MA	MDY	MA	MDY
Aroma								
Cream smell	4-82, 994-1024	2-11, 502-523, 1014-1024	4-380, 728-1024	2-59, 455-571, 967-1024				
Flour smell	60-1000	11-503, 523-1015	-	-				
Appearance								
Yellowness	10-60	-	13-128, 198-417, 942-1015	6-33, 480-546, 992-1021				
Glossiness	2-35, 1007-1024	2-8, 506-520, 1018-1024	3-45, 979-1024	2-45, 242-270, 488-539, 753-784, 1000-1024	488-508, 518-543	62-456, 511-513, 569-968	69-203, 823-987, 1014-1024	2-6, 23-82, 426-595, 942-1001, 1020-1024
Graininess	51-994	14-499, 527-1012	52-115, 209-465, 495-530	-	10-466, 562-671, 920.1015	5-48, 467-559, 977-1021		
Flavour/Taste								
Flour flavour	65-995	14-500, 526-1012	-	-	26-120, 243-266, 322-467, 560-582, 612-656, 920-1006	11-35, 478-549, 991-1013		
Texture/ Mouthfeel								
Viscosity	24-1003	12-502, 524-1014	22-136, 209-306	-	240-787	117-404, 621-911	2-62, 89-99, 227-317, 353-486, 559-670, 984-1024	211-334, 493-531, 711-718, 1006-1024
Smoothness	2-36, 1009-1024	2-6, 507-518, 1020-1024	2-53, 123-149, 997-1024	2-26, 488-539, 1000-1024	2-14, 484-542, 1009-1024	2-6, 63-188, 232-278, 322-456, 508-518, 569-704, 749-794, 836-965, 1020-1024		
Meltdown rate	33-998	14-449, 527-1012	27-116, 210-298	-				
Fatty after mouthfeel	11-59	-	11-119, 926-1010	9-31, 482-544, 994-1017				
Manipulation with spoon								
Non-oral viscosity	27-1004	12-502, 524-1014	23-126, 153-459	-				
Graininess on lid	51-994	14-500, 526-1012	54-113	-	2-187, 231-302, 924-1013	7-34, 479-546, 991-1020		
Metadescriptor								
Creaminess	2-45	-	2-460, 584-642, 961-1024	2-21, 493-533, 1004-1024				

The loading weights reveal how much each X-variable contributes to explaining the response variation along each model component. Hence, the loading weights can be used to find the relationship between the X- and Y-variables. Modelling of creaminess was only possible due to the presence of the 0.3% fat yoghurt without added protein (yoghurt A); removal of this sample led to the collapse of the model. However, this was not the case for any of the other descriptors.

The correlations between the sensory descriptors, as evidenced by CLSM image features, appear from Table 6. The descriptors that correlate in the PLS1 analysis of the AMT spectra are nearly the same as the ones that correlate in the sensory analyses of yoghurt and cream cheese alone. The X-loading weights for the descriptors *creaminess*, *yellow* and *fatty after mouthfeel* are the same, *cream smell*, *smoothness* and *glossiness* somewhat less. X-loading weights for *Graininess*, *graininess on lid*, *flour smell* and *flour flavour* are different from of *smoothness* and *glossiness* and hence negatively correlated. The X-loading weights for *viscosity*, *non-oral viscosity* and *meltdown rate* are also similar and as such positively correlated, but closer inspection revels that they are not mirror images of any of the other descriptors.

Certain features in the images make the prediction of aroma descriptors possible. In the case of *cream smell* this is likely to be related to fat level (which affects microstructure directly). For *flour smell* and *flour flavour* the correlation is likely due to the addition of the commercial milk protein preparation (C).

From the PLS1 analysis it was evident that graininess and viscosity are correlated, but are not one and the same thing. As to graininess, a sample having a fine, close protein network consisting of tiny protein clusters are sensed as being least grainy whereas the samples having a network consisting of large protein clusters and large pores are sensed as grainy. With regards to viscosity, the high viscosity samples are also grainiest. However, the low viscosity yoghurt samples have an overall sparse network with a high degree of porosity.

Qualitatively, the microstructure of the yoghurts with no protein added showed a open, highly porous and regular network, especially the 0.3% fat yoghurt without added protein (yoghurt A). As mentioned previously, an open protein matrix results in a softer and thinner the yoghurt. This agrees with the findings of the present work since yoghurt A is the thinnest yoghurt. As observed in earlier studies, the microstructure of the full-fat yoghurt was denser and more closed than for the yoghurts with a reduced fat content and no addition of protein.

In accordance with the literature, addition of protein increased the connectivity between protein clusters. For the yoghurts containing microparticulated protein, increasing amounts of microparticulated protein lumps were found inside the voids of the protein network, suggesting that the microparticles are not wholly integrated into the network.

Microstructure data - Cream cheese

A general view of the ability of FFT magnitude spectra to discriminate between samples was obtained by ANOVA PLSR: regressing the spectra on the design matrix (Aastveit and Martens, 1986; Martens and Martens, 2001). The total explained variance in Y is 57% for the fire significant dimensions (45, 6, 4 and 2%). Figure 5 and Figure 6 show the first three latent variables, accounting for 55% of the variation in the FFT magnitude spectra.

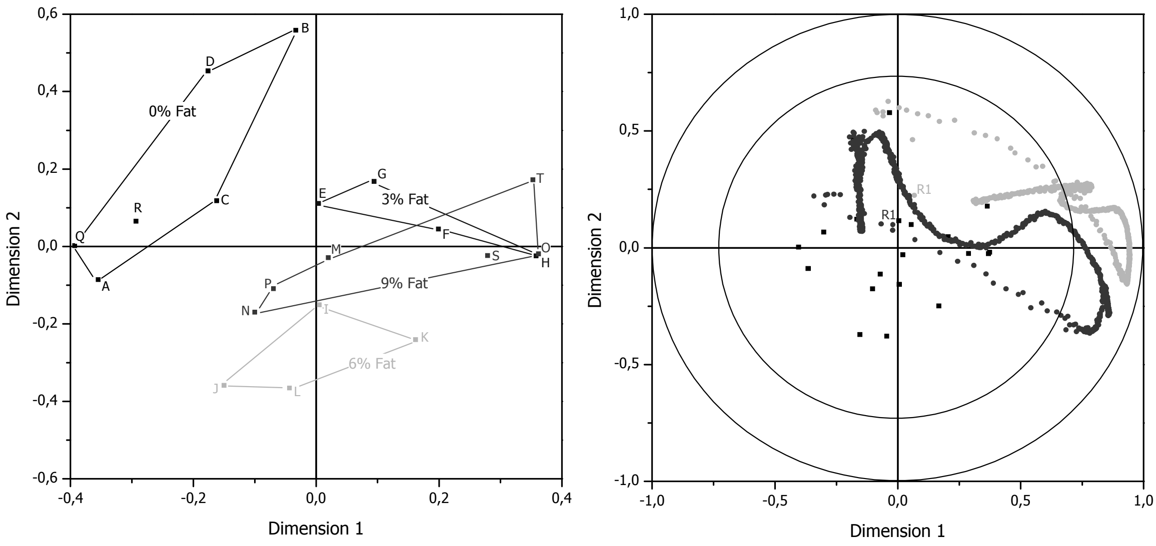

Figure 5. Score and correlation loadings plot from ANOVA PLSR modelling, PC 1 and 2. X-var 5 and 5% / Y-var 45 and 6 (cross-validation over replicate). Dark curve in correlation loadings plot represent red CLSM channel (Nile Red), lighter grey curve represent green channel (Oregon Green).

The score plot of the first two latent variables shows that the samples can be resolved by fat level using the image features alone. This separation is mainly on the first latent variable. Looking at the correlation loadings plot, it can be seen that for the red channel the radius of interest are of a median size whereas for the green channel both median and large radii are of interest. Plotting the first and third latent variables against each other reveals a grouping concerning the production parameters pH value and salt content.

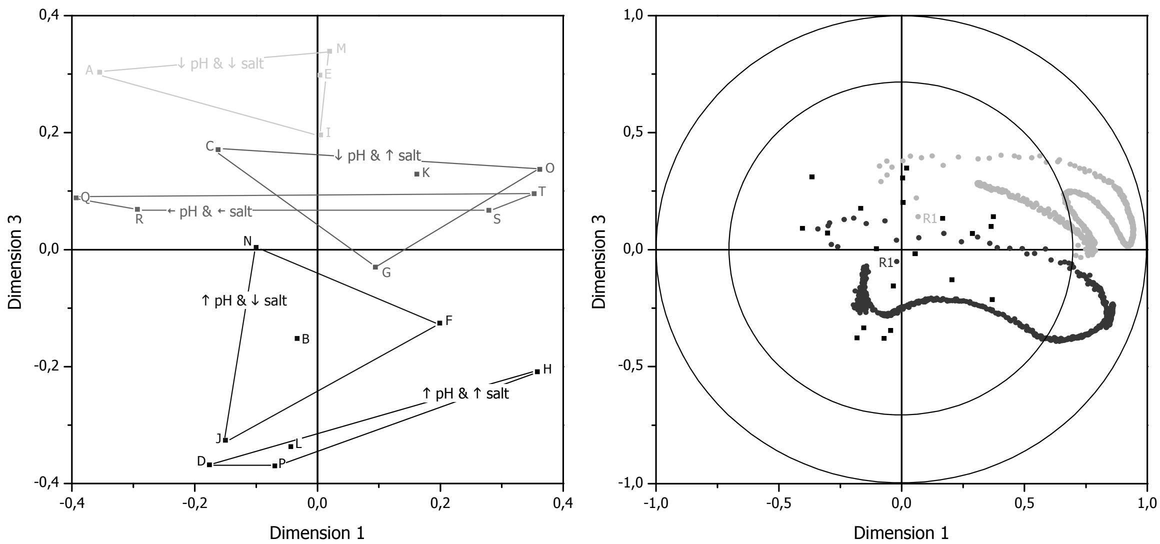

Figure 6. Score and correlation loadings plot from ANOVA PLSR modelling, PC 1 and 3. X-var 5 and 5% / Y-var 45 and 4 (cross-validation over replicate). Dark curve in correlation loadings plot represent red CLSM channel (Nile Red), lighter grey curve represent green channel (Oregon Green).

The score plot shows that the samples with low pH value are located at the upper part of the plot and the high pH samples at the bottom. Within this grouping a another grouping related to salt concentration can be seen, with the upper samples having low salt concentration and the lower samples having high salt concentration. The intermediate points with regards to both pH and salt concentration are located in the middle of the the plot, except for sample G which is overlapping this separation. It should be mentioned that high quality images were difficult to obtain for sample G, which may explain that it is located on the "wrong" side of the replicated, intermediate samples (Q, R, S and T). Turning to the to the correlation loading plot, it is evident that the colour channels are separated, with the green channel being at the score plot the upper part and the red channel at the lower part. This is apparently due to the pH gradient along the third latent variable.

To get a general view of the relationships between the data obtained from the microstructure images (X-variables) and sensory data (Y-variables), PLSR models were developed using leave-one-out cross validation. The analysis was based on the X-loading weights estimated by PLS1 regression (see Table 7).

Table 7. PLS1-modeling of sensory descriptors from FFT magnitude spectra of cream cheese CLSM image, validated by leave-one-out cross-validation.

	Explained X variance (%)	Explained Y variance (%)	Slope	Offset	Correlation	RMSEP	#PLS components
Aroma							
Cream smell	59,13,18	43,38,3	0.75	1.51	0.84	1.02	3
Acidic smell	48,22,18,6	34,38,2,3	0.68	2.26	0.73	0.73	4
Butter smell	59,16,15,5	46,34,6,2	0.81	1.26	0.86	1.00	4
Goat smell	60,46	10,39	0.76	0.72	0.87	0.72	2
Old milk smell	60,14,16	40,25,3	0.55	1.39	0.63	0.72	3
Non-oral manipulation							
Resistance	60,11,18	46,40,2	0.84	1.05	0.88	1.71	3
Appearance							
Greyness	42,33,15	40,26,7	0.60	0.69	0.72	0.31	3
Yellowness	51,24,16	35,32,6	0.56	1.88	0.65	1.04	3
Blueness	59,12,19	39,41,1	0.69	0.53	0.79	0.47	3
Glossiness	59,13,18,1,5	44,40,3,6,0	0.88	0.81	0.92	1.64	5
Grain size	59,11,9	36,37,5	0.65	2.11	0.72	1.60	3
Flavour and taste							
Goat flavour	50,23,17	26,26,3	0.42	1.86	0.53	1.23	3
Butter flavour	60,14,16,2,6	47,32,5,8,1	0.83	1.04	0.87	1.29	5
Cream flavour	59,15,17,4	45,39,4,2	0.88	0.82	0.90	1.19	4
Sour taste	34,38,18	39,20,4	0.45	4.57	0.56	1.89	3
Sweet taste	59,12,19	35,36,1	0.64	1.26	0.74	0.81	3
Texture and mouthfeel							
Smoothness	60,13,17,3,5,1,1	52,31,4,6,2,2,1	0.96	0.23	0.95	1.55	7
Firmness	59,12,18,5,5,1	39,42,2,7,3,2	0.90	0.65	0.91	1.34	6
Flouriness	56,22,12,3,5	40,27,10,12,3	0.80	0.82	0.89	1.20	5
Chalkiness	60,11,18,5,4	54,38,2,2,1	0.96	0.14	0.96	0.88	5
Graininess	60,13,16,3,5,1,1	54,28,4,6,2,3,1	0.97	0.36	0.95	1.70	7
Stickiness	60,14,16,3,5	48,34,5,6,2	0.89	0.50	0.95	1.06	5
Meltdown rate	60,13,17,5,4,1	47,36,5,5,3,1	0.94	0.53	0.96	1.01	6
Astringent	58,15,17,3,5	43,38,5,6,2	0.85	1.07	0.90	1.08	5
Meta-descriptor							
Creaminess	60,15,15,2,5	53,32,5,4,1	0.89	0.80	0.94	1.48	5

The positively correlated X-loading weights for the sensory descriptors are tabulated in Table 8.

Table 8. Positively correlated X-loading weights for the relation between microstructure and sensory descriptors. G=green channel, R=red channel.

Descriptors	Component number					
	1		2		3	
	G	R	G	R	G	R
Aroma						
Cream smell	30-562, 619-722	12-417	-	12-176, 342-417, 544-636	424-563, 619-722	1-24, 312-722
Acidic smell	1-32, 367-390, 548-624	1-12	1-722	8-24, 186-285	1-30, 360-420, 534-632	1-24, 300-722
Butter smell	28-567, 612-722	16-409	-	16-94, 349-409, 548-632	426-552, 620-722	34-89, 333-722
Goat smell	1-18	1-10, 507-522, 670-722	1-722	1-10, 456-522, 675-722		
Old milk smell	1-16	1-10	1-722	-	1-48, 354-458	1-16, 296-328, 390-581, 606-722
Non-oral manipulation						
Resistance	1-19	1-0,463-534, 649-722	1-722	247-321, 447-527, 662-722	1-168	1-75,117-308
Appearance						
Greyness	1-27, 559-617	423-550, 631-722	1-54	-	1-722	79-341, 448-522, 679-722
Yellowness	72-346, 415-540, 634-722	16-674	-	1-5, 346-448, 528-664	72-262	25-184, 346-384
Blueness	1-20	679-722	1-722	492-514, 698-722	1-90, 147-193	1-25, 148-264, 392-722
Glossiness	32-548, 624-722	14-417	-	14-141, 341-417, 547-633	242-326, 425-549, 624-722	307-722
Grain size	1-20	1-10, 512-528, 653-722	1-722	1-10, 465-522, 674-722	1-204	1-88, 144-192, 528-630
Flavour and taste						
Goat flavour	1-29	-	1-722	231-325	1-29, 374-722	300-335, 390-567, 612-722
Butter flavour	30-564, 616-722	15-410	-	15-163, 345-410, 550-631	253-316, 425-544, 624-722	34-69, 330-722
Cream flavour	15-722	14-399	-	14-95, 352-396, 559-619	469-533, 655-722	1.19, 87-113, 281-722
Sour taste	1-40, 352-410, 540-632	1-10	1.722	1-340	1-23, 379-434, 526-649	1-26, 384-722
Sweet taste	31-556, 620-722	16-422, 566-616	-	16-162, 340-422, 541-639	1-12, 410-722	306-722
Texture and mouthfeel						
Smoothness	27-575, 608-722	11-412	-	11-182, 342-412, 548-633	269-312, 415-562, 617-722	323-722
Firmness	1-21	1-9, 455-534, 648-722	1-722	223-328, 444-527, 660-722	1-145, 591-597	1-46, 114-317
Flouriness	1-21	1-9, 418-558, 625-722	1-722	200-345, 418-540, 640-722	1-97,567-608	214-298
Chalkiness	1-17	1-7, 454-540, 640-722	1-87, 214-722	246-327, 437-533, 652-722	1-161	1-57, 151-265
Graininess	1-16	1-10, 448-547, 634-722	1-88, 233-722	260-323, 433-538, 648-722	1-68	-
Stickiness	1-18	437-547-547, 635-722	1-96, 226-722	232-332, 429-536,648-722	1-140	1-10, 159-270
Meltdown rate	28-574, 608-722	10-416, 576-609	-	10-106, 347-416, 544-637	230-329, 404-564, 617-722	342-722
Astringent	1-21	440-548, 634-722	1-722	232-331, 434-535, 649-722	1-136, 590-597	155-251
Meta-descriptor						
Creaminess	28-568, 615-722	14-408	-	14-175, 346-408, 553-630	440-544, 627-722	35-114, 312-722

Here we notice that the X-loading weights for *creaminess* are almost the same as those of *butter flavour*, *cream flavour*, *glossiness*, *smoothness* and *meltdown rate*. *Smell of cream* and *smell of butter* are close by. Finally *sweetness* and *yellowness* has some similarities to *creaminess*. Again, *graininess* is negatively correlated to *creaminess*. *Hand resistance*, *firmness*, *chalkiness*, *stickiness* and *flouriness* are positively correlated to graininess whereas *smell of goat* and *astringent* has some similarities. *Flavour of goat*, *smell of old milk*, *smell of acidic*, *sourness*, *grain size*, *blueness* and *greyness* all correlate differently to some of the same X-loading weights as *graininess* do.

The sensory analysis showed a close correlation between many of the descriptors. Since certain features in the images relates to some of the textural descriptors and these correlates to smells, flavours and tastes make the prediction of these possible. For example in the case of *cream smell* it is likely to be a structure relating to fat content and for *acidic smell* and *sour* properly the effect of the pH value on the colouring of the samples as the dye Oregon Green® was pH sensitive.

Conclusion

It was possible, by application of AMT anf FFT, respectively, to discriminate between the microstructure in both the yoghurts and cream cheeses and reveal some of the same correlations between visual, textural and olfactory related descriptors as seen in the sensory analysis alone.

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Sensory and rheological characterization of acidified milk drinks

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Abstract

A set of seventeen acidified milk drinks, of which eight were drinking yoghurts (made by dilution of a yoghurt base) with 3-8% milk solids non fat (MSNF) and the remainder milk-juice drinks (made from fruit concentrate and reconstituted milk powder) with 3% MSNF were submitted to descriptive sensory analysis and rheological characterization. The drinks were stabilized with pectin and/or carboxy methyl cellulose, CMC, (0-0.5%). The sensorially perceived *Creaminess* was found to depend linearly on *Oral viscosity* whereas the relationship between Creaminess and Smoothness depended on the level of MSNF. Drinking yoghurt stabilized using pectin were found to be shear-thinning whereas pectin-stabilized milk-juice drinks as well as either category of acidified milk drink stablilized by CMC were found closely approximate Newtonian behaviour. Viscometry data could only predict sensory viscosity moderately well, presumably due to the different functional properties of pectin and CMC.

Key words

Acidified milk drinks, sensory, rheology, pectin, CMC

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Introduction

Acidified milk drinks are a diverse group of beverages including drinking yoghurts and milk/juice drinks (Nakamura, Yoshida, Maeda and Corredig, 2006). A common denominator of these products is their low pH and low viscosity, which results in sedimentation problems due to aggregation of milk protein (Amice-Quemeneur, Haluk, Hardy and Kravtchenko, 1995). Drinking yoghurts are made by diluting a fermented yoghurt base with water (and often fruit concentrate as well) whereas milk-juice drinks are made from diluted fruit concentrate and milk powder. A special variety of acidified milk drink is the Turkish product *ayran*, which contains salt.

To prevent or reduce the aggregation of milk protein (i.e. casein) acidified milk drinks are commonly stabilized with high methoxy pectin. In dilute acidified milk systems pectin adsorbs onto the casein micelles as the result of an electrostatic interaction (Tuinier, Rolin, & de Kruif, 2002) and the mechanism of stabilisation of AMDs has been proposed to involve adsorption of pectin chains onto the micellar surface mediated by the charged blocks of the pectin molecule, while the uncharged domains form loops that extend into the solution and cause repulsive interaction between the micelles at low pH much as κ-casein does at pH 6.7 (Tromp et al, 2004).

A weak gel network, necessary for long-time stability, has been found to be formed in stabilised acidified milk drinks, as evidenced by an increase in elastic modulus subsequent to shearing (Sedlmeyer, Brack, Rademacher and Kulozik, 2004). Tromp, de Kruif, van Eijk and Rolin (2004) found that up to 90% of the pectin added as a stabiliser to AMDs did not directly interact with the milk protein particles. They proposed that complexes of casein micelles with adsorbed pectin form a self-supporting network which provide the stability in AMDs. The non-adsorbed pectin in the serum is then linked to this network but not necessary for the stability *per se*.

The protein-pectin interaction, and thereby the stability of AMDs, depends on pH, the concentration and type of pectin used, the concentration of casein and the ionic strength as well as on homogenisation and thermal treatment during processing (Glahn, 1994).

Model studies of acidified milk drinks have been carried out using reconstituted skimmed milk powder and the acidulant glucono-δ-lactone (Lucey, Tamehana, Singh and Munro, 1999). The rheological properties of these models were found to be slightly different from those of acidified milk drinks made by fermentation, in that the Herschel-Bulkley flow behaviour index (n) was lower in drinks made using GDL, whereas the consistency index (K) and the yield stress (σ_0) were higher. The effect of pasteurization temperature, storage temperature and pectin concentration on syneresis, rheological properties, particle size and microstructure has been studied (Lucey *et al*., 1999). Syneresis could be modelled satisfactorily (R^2 = 0.88), while n and particle size were less well predicted (R^2 = 0.51 and 0.63, respectively).

Sensory studies of acidified milk drinks have been limited to hedonic evaluations by untrained panellists (Koksoy and Kilic, 2004; Penna, Sivieri and Oliveira, 2001). Acceptability of commercial acidified milk drinks has been found to be correlated positively to flow behaviour index n and consistency index K (Penna *et al.*, 2001). In ayran, high levels of added hydrocolloids have been found to be detrimental to acceptability (Koksoy and Kilic, 2004).

Creaminess has been found to be correlated to consumer acceptance in a wide range of dairy products, including fresh and reconstituted milks and creams (Richardson-Harman *et al*., 2000), yoghurts (Folkenberg and Martens, 2003; Ward, Koeferli, Schwegler, Schaeppi and Plemmons, 1999) and ice cream (Lähteenmäki and Tuorila, 1994). The purpose of the present work was to shed light on the sensory and rheological properties of this category of dairy products, with a particular view to creaminess.

Materials and methods

Acidified milk drinks

Seventeen acidified milk drinks were manufactured according to an incomplete factorial design. Several factors were varied: Milk Solids Non-Fat level (MSNF: 2% and 8.5%); Acidification method (Lactic acid bacteria (yoghurt) and citric acid to final pH=3.95); addition of two hydrocolloids (GENU® Pectin type YM-115-L and CEKOL® cellulose gum type HVD) at different levels, either alone or in combination. The amount of sugar (food grade sucrose): 8% and raspberry concentrate (65°Brix): 1% was kept constant in all 17 samples. The reason for the selection of factors and levels was to achieve commercial relevance as well as a wide span of sensory properties. Products with lactic fermentation (drinking yoghurts) were made from a yoghurt base (based on reconstituted skim milk powder); mixed with sugar, water, hydrocolloid solution and raspberry concentrate to achieve the desired levels. Products acidified with citric acid (milk-juice drinks) were made from reconstituted skim milk; mixed in the same manner as decribed above. For both types final pH was adjusted to 3.95. The mixes were homogenized at 60°C, 180/50 bar, pasteurized at 90° for 15 seconds, cooled and kept at refrigerator temperature until instrumental and sensory measurements.

Sensory analysis

Descriptive sensory analysis was performed by a panel of trained panellists. All panellists were selected according to international standards (ISO-8586-1 1993). Twenty sensory descriptors were developed by consensus during training sessions (3, duration approximately 1.5 hours), using refererence samples where feasible. Table 1 lists descriptors, abbreviations, definitions and reference material. In addition, the descriptor *Creaminess* was evaluated without prior consensus among panellists, *i.e.* each panellist used his or her own concept of *Creaminess*. Sensory analysis took place in a sensory laboratory complying with international standards for test rooms (ISO-8589

1988). Samples were scored on a computer screen using a 15 cm unstructured scale; a computerized score collection software (FIZZ, BIOSYSTEMES, Couternon, France) was employed. The scales were anchored with "a little" and "a lot" ("*lidt*" and "*meget*", in Danish), except for the viscosity descriptors, for which the terms "thin" and "thick" ("*tynd*" and "*tyk*") were used.

Rheology

Steady shear viscometry was performed in triplicate at 12ºC using a concentric double gap (24/27 mm) measuring system in a Bohlin C-VOR (Malvern Instruments Ltd., Malvern, Worcestershire, UK) controlled stress rheometer. Shear stress was measured at 30 logarithmically spaced shear rates in the range 0.1-100 s^{-1}. The flow curves from viscometry were fitted to the Power Law model using non-linear regression.

Data analysis

Initially, univariate analysis of variance (ANOVA) and multivariate data analysis (ANOVA-Partial Lest Squares Regression (ANOVA-PLSR)) were applied to the sensory data. Mixed model ANOVA for individual descriptors was performed with products (n=17) as fixed factors and panellists (n=10) as random factors. This method is commonly applied for data from descriptive analysis (Næs and Langsrud, 1998). For descriptors with non-significant Product X Panellist interaction effects, interactions were omitted in a second analysis. Non-significant descriptors were omitted from further analysis. Confidence intervals at 95% level (CI95%) were estimated based on Mean Square Error. ANOVA-PLSR is a multivariate regression method where the effect of design factors on the response variables (here: the sensory descriptors) is evaluated (Martens and Martens, 2001; Martens and Martens, 1986). The method avoids multicollineariety problems by modelling latent variables (LV) representing the main variation found to be common for the variables. The method evaluates effects of the experimental design variables on sensory properties. We have used it here as a graphical alternative to ANOVA (Aastveit and Martens, 1986).

For multivariate analyses cross validation was performed, leaving out one replicate at a time (Martens and Næs, 1989). Jack-knifing with replicates served as the validation tool for all multivariate analysis, comparing the perturbed model parameter estimates from cross-validation with the estimates for the full model (Martens and Martens, 2000). For multivariate data analysis, data were averaged over panellists, and those data were used for analysis of product properties and relationships with instrumental measurements. Sensory-instrumental relations were modelled by uni- and multivariate techniques, using The Unscrambler Ver. 9.2 (CAMO Process AS, Oslo, Norway).

Results and discussion

Sensory data

The results from ANOVA showed that 20 of the descriptors had significant differences among the samples. The descriptor *Curtains* was found not to discriminate significantly between the products, and was thus excluded from further analysis. Table 2 lists mean, *p*-value from ANOVA and confidence intervals (CI 95%) for all significant descriptors. Figure 1 displays results from ANOVA-PLSR in a geometrical manner. Figure 1A shows the distribution and differences among products, also indicating the different factors and levels in the object labels. Product differences are referred to along with the explanations in the text. Figure 1B shows the correlation loading plots of the sensory descriptors from the first two of the three significant latent variables (explaining 76, 8 and 4 % of the variation in sensory data, respectively, data averaged over panellists). Close scrutiny of Table 2 and The Jack-knife pertubation plots and estimation of model parameter stability from ANOVA-PLSR (not shown) revealed that all 17 samples were significantly different in their sensory properties. Thus, the descriptive analysis demonstrated and explained the effects of all experimental factors on sensory properties. The grouping and orientation of the descriptors in Figure 1B show that the first latent variable was closely related to the highly interesting descriptor *Creaminess* and also to a number of other descriptors encompassing both appearance, aroma, taste, flavour and texture. On the right side of Fig 1B, a group of correlated descriptors was located. Those best explained all relate to texture: *Viscosity (Visual* and *Oral)* and *Resistance*. Among the flavour descriptors are *Cream flavour*, *Buttermilk aroma* and *Buttermilk flavour*, and the somewhat less explained descriptor *Citrus flavour*. *Fatty after mouth feel* was also correlated to this group, though slightly less, and a highly negative correlation was apparent with *Transparency*, *Sweet, Boiled milk aroma* and *Boiled milk flavour*. The second latent variable was spanned by differences in *Smoothness* and to some degree also *Colour*. A third latent variable, also related to differences in *Smoothness*, was necessary to fully describe all systematic sensory variance among the 17 samples. The samples with highest intensity of *Creaminess* were, not surprisingly, the three samples with high MSNF level. The samples with a low MSNF level and a relatively high *Creaminess* were those with the highest *Viscosity* (Figure 1A and Table 2), i.e. the samples with the highest CMC content, either alone, or in a mixture with pectin (samples 2lacC5, 2citC5 and 2citP2C4). They possessed a combination of high *Smoothness* and medium *Viscosity* and had a significantly higher *Creaminess* than the remaining samples with a low MSNF level. This is not immediately apparent from the relationships displayed in Figure 1B. Figure 2 shows the direct relationship between *Smoothness* and *Creaminess* for both low and high levels of MSNF. It is evident that the difference in MSNF resulted in two distinctly different types of relationships. For the low level of MSNF, *Smoothness* and *Creaminess* were positively correlated, whilst the relation was negative at the high level of MSNF. We suggest two reasons for these differences: 1) The samples made with high level of MSNF have have a higher intensity in dairy flavours which enhance *Creaminess* (*Buttermilk* and *Cream flavour*) as well as a lower intensity in dairy flavours that contribute to decrease *Creaminess* (*Boiled milk flavour*), as apparent from Table 2; 2) At higher level of *Viscosity*, its contribution to *Creaminess* exceeds that of *Smoothness*, so samples with low *Smoothness* can still possess a very high *Creaminess.* Detailed studies systematically and independently varying levels of *Viscosity*, *Smoothness* and *e.g. Cream flavour* will, however, be necessary to disentangle the contribution of different sensory properties to *Creaminess*.

Rheological data

Power law parameters *K* and *n* are tabulated in Table 3. Some of the flow curves fitted poorly to the Power law model. Overall, the drinking yoghurts appeared to be more viscous (higher *K*) and less Newtonian (lower *n*) than the milk-juice drinks. As expected, *K* increases with increasing milk solids and hydrocolloid dosage. Flow curves for drinking yoghurts and milk-juice drinks with 0.3% pectin and without CMC were clearly distinguishable (Figure 4); shear-thinning is evident in the drinking yoghurt while the milk-juice drink is essentially Newtonian. By contrast, flow curves for drinking yoghurts and milk-juice drinks with 0.3 % CMC and without pectin coincided almost completety except at very low shear rates (Figure 5).

Pectin is thus capable of inducing an ordered structure to drinking yoghurt but not milk-juice drinks; the only effect of CMC is to increase the viscosity - it does not make the structure any more ordered. This ability of pectin to induce formation of a weak network structure in AMDs is in accordance with earlier studies (Sedlmeyer *et al*., 2004; Tromp et al, 2004). CMC, which is also an anionic hydrocolloid, is know to interact with casein micelles (Everett & McLeod, 2005) and to form soluble complexes with milk protein at low pH (Doublier et al, 2000). Our rheological results indicate that milk protein aggregates stabilised by CMC in AMDs do not interact as extensively with each other or with non-absorbed polysaccharide as does high methoxy pectin. A more elaborate multivariate analysis using ANOVA PLSR on the individual raw flow curves showed that the pair 2lacP3 (drinking yoghurt)/2citP3 (milk-juice), both stabilized by 0.3% pectin, could be clearly distinguished rheologically, whereas the equivalent pair (2lacC5/2citC5) stabilized by 0.3% CMC was indistinguishable from each other. Looking closer at the matching sample pairs of drinking yoghurt and milk-juice drinks with identical levels of MSNF, pectin and CMC (Figure 6), the pair 2lacP3/2citP3 was barely distinguished sensorially with respect to *Oral viscosity* while 2lacC5/2citC5 was indistinguishable. So, even if we could clearly measure a difference between drinking yoghurt and milk-juice drink at 0.3% added polysaccharide, the panel could hardly sense it.

The Power law consistency index *K* was able to predict *Oral viscosity* moderately well (r = 0.70). However, this was essentially spanned by the samples with 8% MSNF (8lacP4, 8lacP5 and 8lacC5); within the remaining samples with 3% MSNF the correlation was r = 0.00. *Oral viscosity* was predicted moderately well by PLS regression on the shear stress data matrix with just one latent variable (R^2 = 0.70). The predictive ability of these models was clearly inferior to other, uni- and multivariate models of sensory viscosity of dairy products (Janhøj, Petersen, Frøst and Ipsen, 2006b; Janhøj, Frøst, Andersen, Viereck, Ipsen and Edrud,, 2006a). The reasons seems to be the different functional properties (and mode of interaction with milk protein) between of pectin and CMC, which influence the rheological properties in different ways. Another possible problem is turbulence at higher shear rates.

Conclusions

Creaminess in acidified milk drinks appears to be largely determined by sensory viscosity. However, sensory viscosity is only predicted moderately well from rheological data.

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Tables

Table 1: Sensory descriptors, their definitions and reference material, if used.

Descriptor	Definition	Recipe for reference material
Appearance		
Glass coating	Amount of milk drink coating glass after swirling glass thoroughly	
Curtains	How cohesive was the glass coating	
Transparency	Transparency of the sample at the edge of the glass tilted approximately 45°	
Viscosity	Measured during swirling of glass (thin – thick)	
Colour	Colouration (white to red)	
Aroma		
Buttermilk	Intensity when sniffed	Buttermilk
Raspberry	Intensity when sniffed	0.5 L 0.5% fat milk added 30 ml organically produced raspberry cordial mixer
Boiled milk	Intensity when sniffed	0.5 L 3.5% fat milk + ½ caramel roll + 100 g parsnip boiled until the caramel melted and the parsnip was soft. Sieved and cooled
Taste		
Sweet	Intensity	0.5 L 0.5% fat milk added 45 ml food grade sucrose
Flavour		
Buttermilk	Intensity when tasted	Buttermilk
Raspberry	Intensity when tasted	Same as for raspberry aroma
Cream	Intensity when tasted	38% fat cream
Citrus	Intensity when tasted	A small piece of lemon
Boiled milk	Intensity when tasted	Same as for boiled milk aroma
Texture		
Resistance	When sucking sample through a straw.	
Viscosity (Viscosity-O)	Measured in mouth (thin – thick)	
Smoothness	Measured in mouth	
Floury	Measured in mouth	0.5L 0.5% fat milk added 30 ml wheat flour
Astringent	Lack of lubrication measured between inner side of incisors and tongue	Quark
Fatty after-mouthfeel	Measured in mouth	38% fat Cream
Meta descriptor		
Creaminess	Idiosyncratic definition, not discussed	

Table 2: Product abbreviations and experimental design for acidified milk samples. Mean, *p*-value and confidence intervals (95% level; CI 95%) for all sensory descriptors.

Product abbreviation					Appearance				Aroma			Taste
	MSNF (w/w %)	Acidification method	Pectin Level (w/w %)	CMC Level (w/w %)	Glass Coating	Transpa-rency	Visual Viscosity	Colour	Butter-milk	Raspberry	Boiled milk	Sweet
8lacP4	8.5	Lactic acid bacteria	0.4		13.0	1.4	10.4	4.4	10.1	7.2	3.3	8.3
8lacP5			0.5		13.4	1.1	10.7	3.3	9.8	7.4	3.5	7.3
8lacC5				0.5	12.2	1.4	9.3	3.7	9.6	7.8	4.1	8.3
2lacP3	2		0.3		5.6	6.7	3.4	5.8	5.8	7.6	6.2	10.5
2lacP4			0.4		6.6	6.3	4.3	6.7	5.3	8.6	5.5	10.7
2lacP5			0.5		7.5	5.7	4.9	7.9	6.3	7.8	4.7	10.3
2lacC3				0.3	7.1	5.8	4.6	7.4	5.9	8.0	4.7	9.4
2lacC5				0.5	9.2	4.9	6.4	9.5	6.0	8.3	5.4	10.2
2citP2	2	Juice + citric acid	0.2		2.9	7.4	2.6	10.5	4.1	8.7	6.8	13.2
2citP3			0.3		4.5	6.5	2.9	4.0	4.7	8.8	5.8	10.4
2citP4			0.4		4.3	6.1	4.0	11.5	4.0	9.3	5.2	10.7
2citP5			0.5		5.4	6.4	4.2	10.4	4.7	8.2	5.7	10.1
2citC3				0.3	3.3	6.2	3.7	6.6	5.1	7.8	5.5	10.2
2citC5				0.5	4.3	5.8	5.2	7.5	4.6	8.3	5.3	9.5
2citP2C2			0.2	0.2	3.7	5.5	3.5	5.2	4.6	8.5	4.9	10.1
2citP2C3			0.2	0.3	5.9	4.7	5.4	4.7	4.8	7.6	5.2	8.4
2citP2C4			0.2	0.4	6.6	4.8	5.9	7.8	4.7	8.5	6.0	9.7
p-value					<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	0.007	0.003	<0.0005
CI 95%					0.8	0.5	0.7	0.5	0.8	0.7	0.9	0.7

Table 2 continued

	Flavour					Texture						Meta-descriptor
Product abbreviation	Butter milk	Raspberry	Cream	Citrus	Boiled Milk	Resistance	Oral Viscosity	Smoothness	Floury	Astringent	Fatty after mouth feel	Creaminess
8lacP4	10.5	7.8	4.1	5.7	4.1	11.6	11.4	4.8	5.4	8.5	3.7	8.1
8lacP5	10.8	7.8	3.9	5.8	3.5	11.9	11.3	3.9	5.1	8.8	4.3	8.9
8lacC5	10.0	8.1	4.5	5.8	4.0	9.4	8.8	5.2	4.4	8.0	3.5	7.3
2lacP3	3.2	7.2	1.9	3.3	9.4	2.5	2.2	5.2	1.9	3.5	1.7	1.5
2lacP4	3.1	8.0	2.1	3.2	7.8	3.2	3.0	6.0	1.7	4.6	2.4	2.6
2lacP5	3.0	7.2	2.0	3.4	6.4	5.2	4.6	6.4	2.0	3.3	3.3	3.3
2lacC3	3.9	7.6	2.1	4.0	4.2	3.6	3.4	5.2	1.8	4.4	2.1	2.0
2lacC5	4.5	7.7	3.0	4.4	4.8	8.1	6.9	7.4	3.2	4.4	4.5	5.6
2citP2	2.5	7.8	1.4	3.4	9.0	2.3	2.0	5.0	1.6	4.9	1.3	1.2
2citP3	2.7	7.5	1.8	3.6	4.5	1.9	1.6	3.8	1.7	4.5	1.5	1.2
2citP4	3.1	8.6	1.6	4.6	5.3	3.3	2.4	5.6	1.5	4.1	2.2	1.6
2citP5	2.8	7.6	1.7	4.1	5.1	5.1	4.2	5.9	1.9	4.0	2.9	2.7
2citC3	3.1	8.0	1.6	4.8	4.5	3.1	3.1	5.9	1.5	4.3	2.3	2.2
2citC5	3.8	7.7	2.1	4.9	4.9	7.1	6.3	7.9	2.4	5.3	4.1	4.6
2citP2C2	3.2	8.6	1.8	4.6	5.1	3.5	2.9	5.1	1.8	4.2	1.8	2.0
2citC3	3.2	7.4	1.7	4.2	5.4	5.8	5.1	7.1	2.5	4.9	2.9	3.9
2citP2C4	4.0	8.6	2.7	5.1	5.0	8.5	7.5	8.5	3.2	5.0	4.2	5.5
p-value	<0.0005	0.026	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
CI 95%	0.6	0.6	0.5	0.7	0.9	0.6	0.6	0.7	0.5	0.7	0.7	0.7

Table 3: Power law parameters for flow curves (means of replicates).

*Could not be fitted to the Power law model.

Product abbreviation	MSNF (w/w %)	Acidification method	Pectin Level (w/w %)	CMC Level (w/w %)	Power law parameters		
					K	*n*	R^2
8lacP4	8.5	Lactic acid bacteria	0.4	0	0.1012	0.7544	0.99
8lacP5			0.5	0	0.1054	0.7487	0.99
8lacC5			0	0.5	0.1526	0.6725	0.94
2lacP3	2		0.3	0	0.0708	0.6548	0.93
2lacP4			0.4	0	0.0189	0.7452	0.88
2lacP5			0.5	0	0.0437	0.6161	0.96
2lacC3			0	0.3	0.0156	0.4234	0.81
2lacC5			0	0.5	0.0280	0.8185	0.95
2citP2	2	Juice + citric acid	0.2	0	0.0407	0.8043	0.74
2citP3			0.3	0	*	*	*
2citP4			0.4	0	0.0166	0.7559	0.82
2citP5			0.5	0	0.0265	0.7560	0.92
2citC3			0	0.3	0.0108	0.7735	0.62
2citC5			0	0.5	0.0303	0.8366	0.96
2citP2C2			0.2	0.2	0.0128	0.7953	0.57
2citP2C3			0.2	0.3	0.0232	0.8230	0.94
2citP2C4			0.2	0.4	0.0349	0.8447	0.97

Figure captions and figures

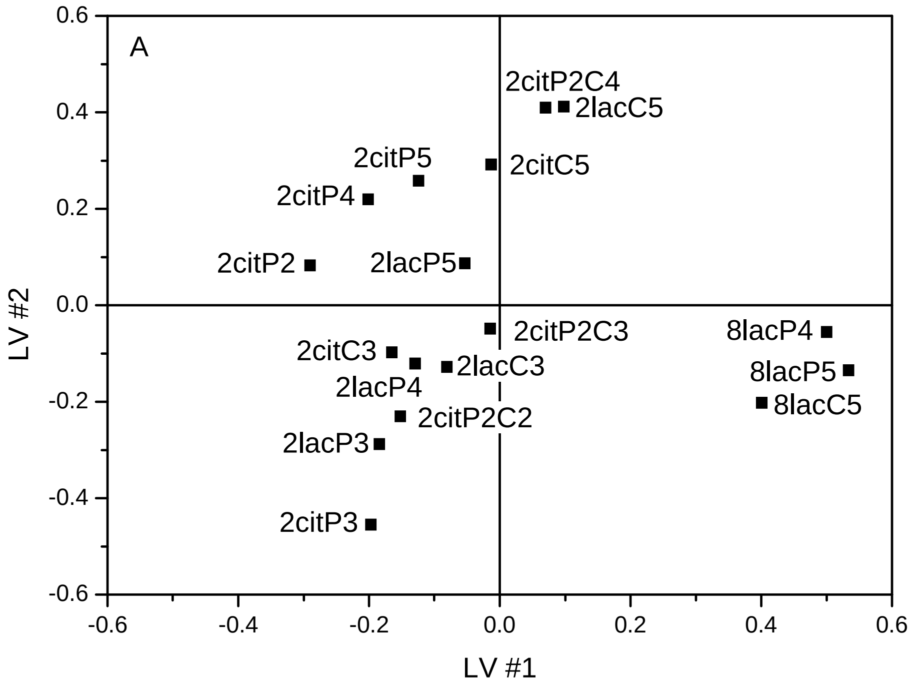

Figure 1A: Score plot from Anova-PLSR showing how experimental design and sensory descriptors correlate with latent variables.

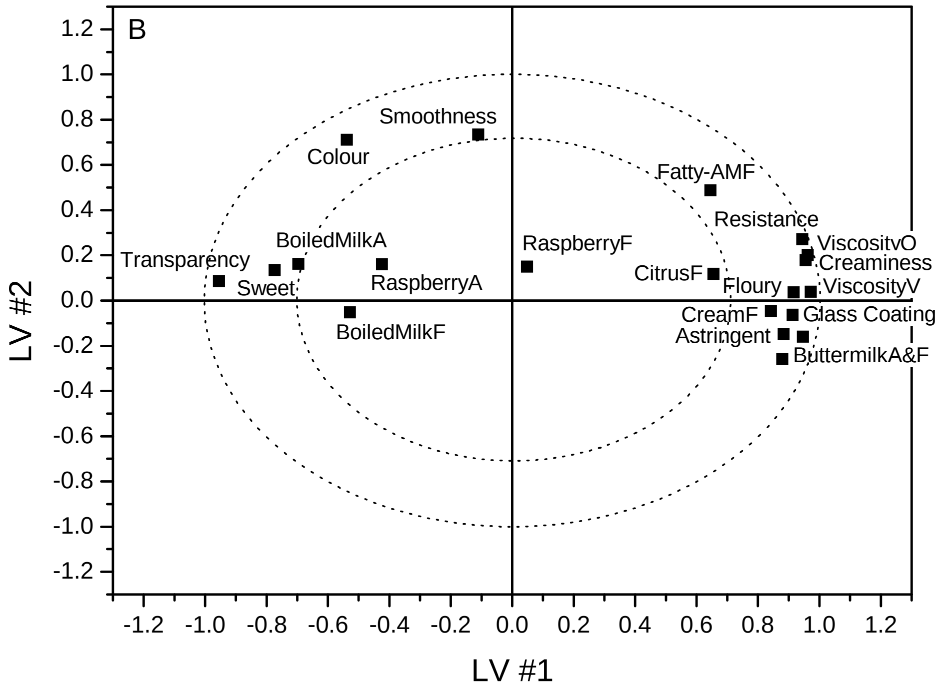

Figure 1B: Correlation loading plot. Correlation loading plot, showing interrelationships among sensory descriptors and to latent variables. The inner and outer circles represent 50% and 100% explained variance, respectively.

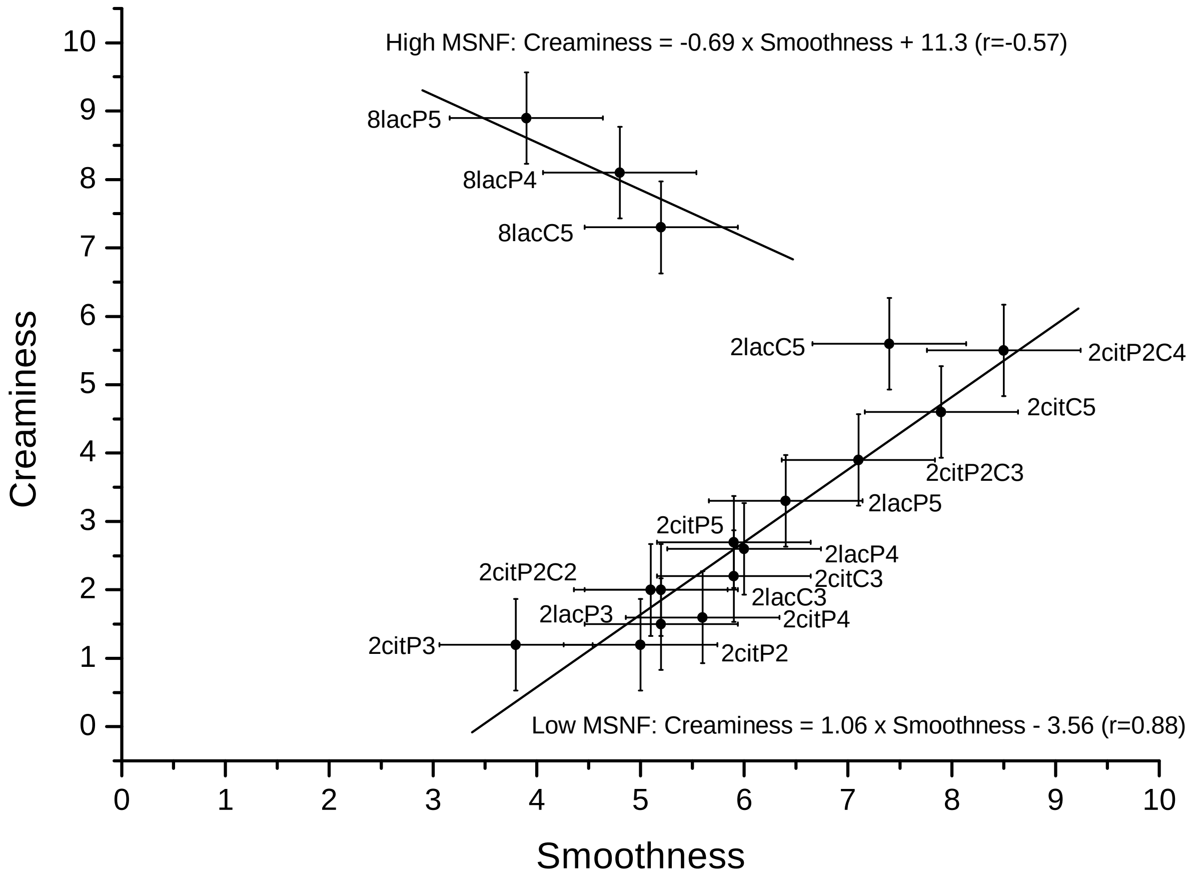

Figure 2: Relationships between Smoothness and Creaminess, specified for High and Low MSNF level groups of samples.

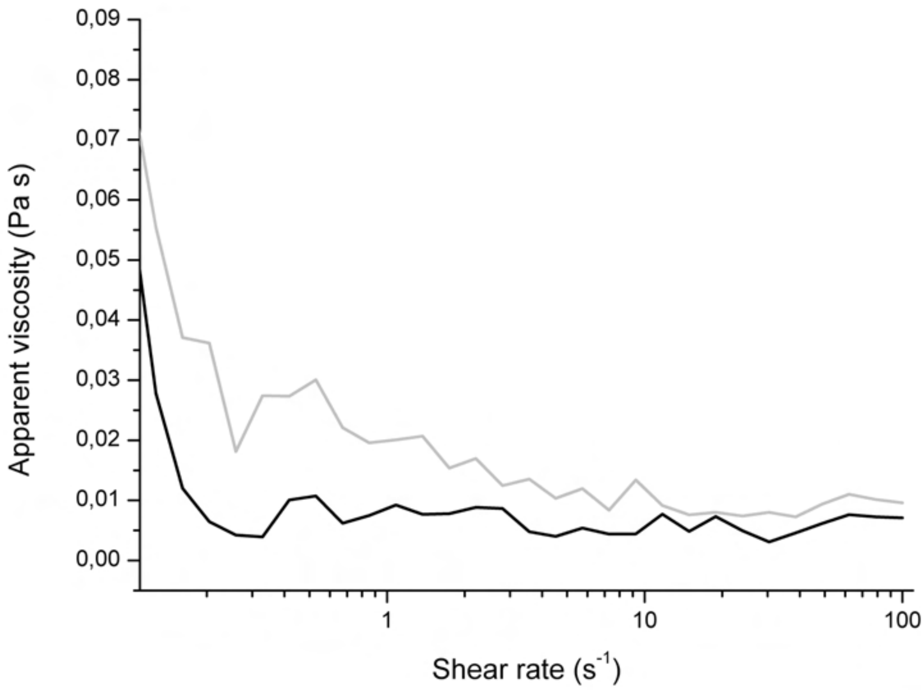

Figure 4: Flow curves for drinking yoghurt (2lacP3; grey line) and milk-juice drink (2citP3; black line), both with 0.3% pectin and 0% CMC.

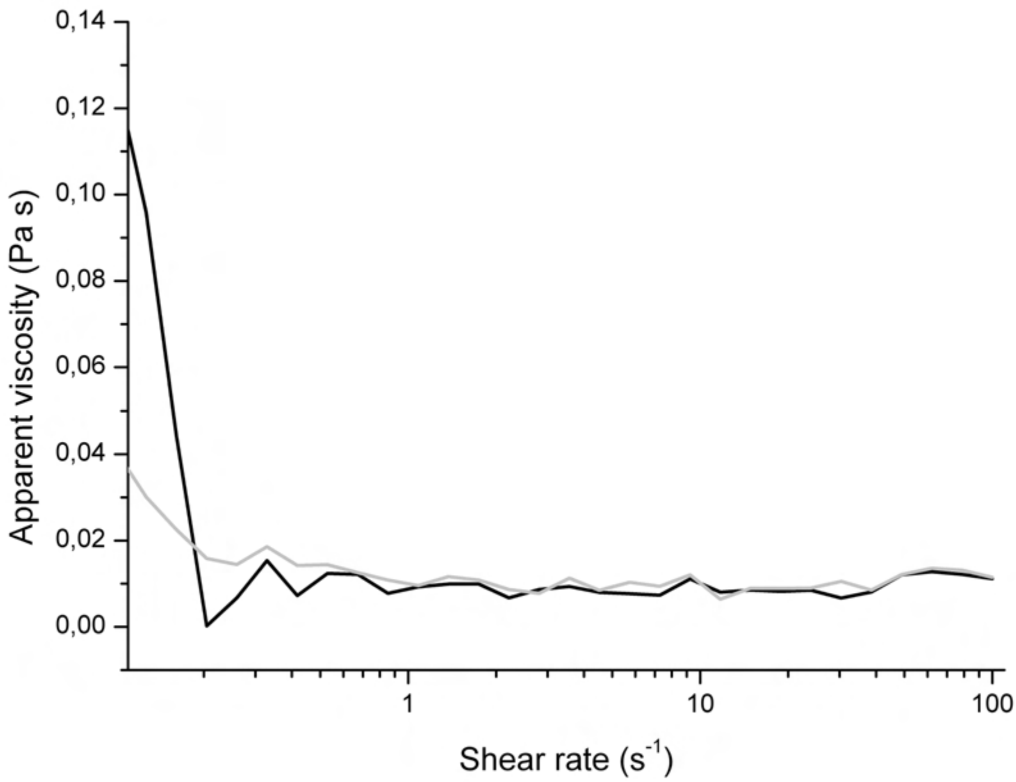

Figure 5: Flow curves for drinking yoghurt (2lacC3; grey line) and milk-juice drink (2citC3; black line), both with 0.3% CMC and 0% pectin.

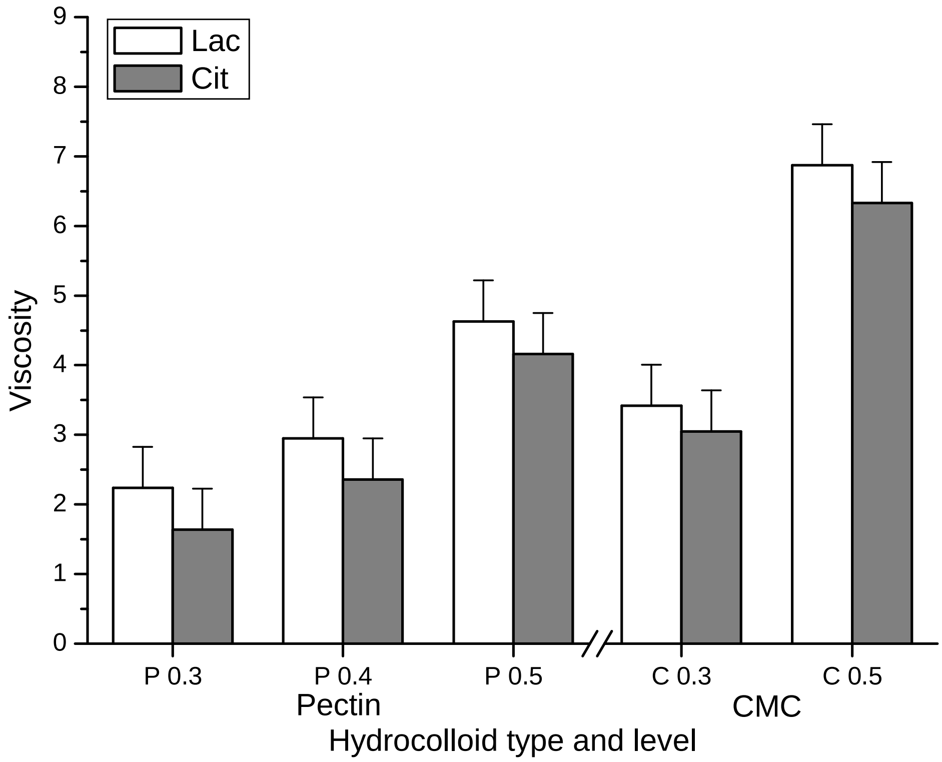

Figure 6: Oral viscosities of pairs of drinking yoghurts (Lac) and milk-juice drinks (Cit) with equal dosage levels of pectin and CMC.